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INHERITANCE IN TETRAPLOID DATURAS

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Introduction

Tetraploidy is a term applied to a doubling of the number of chromosomes in the constituent cells of a plant or animal. By some writers, for example Gates (**14**), a species which has twice as many chromosomes as a closely related form is called tetraploid. The writers, however, feel strongly the desirability of confining the term to a doubling of homologous chromosomes, as is done by Morgan (**21**). In the narrower sense in which we shall use the word, therefore, a tetraploid plant will have in each somatic cell four homologous chromosomes in each chromosomal set, whereas a diploid plant has but two and a haploid plant (**8**) or haploid stage has but one in each set. The mere fact that a plant has twice as many chromosomes as a related form, although affording presumptive evidence, will not of itself justify one, we believe, in calling it a tetraploid. The increase in number may have been brought about by fragmentation or by transverse division of the individual chromosomes, with the result that the four members of a given set will not all be homologous. Before the term tetraploid can properly be applied, therefore, there must be evidence from the cytology or from the breeding behavior to indicate that the increase in number has been brought about by a doubling of the homologous chromosomes in each set. That a mere numerical count of the individual chromosomes may not enable us to classify properly a plant

is shown by the fact that we have found individuals of *Datura* in which the presence of an extra chromosome in one set was numerically compensated by a deficiency in another set. Thus, of two plants from our cultures, each of which had a total of 48 chromosomes in their somatic cells (the same number found in true tetraploids), one appears to have been a chromosomal mutant of the type $(4n+1-1)$ and the other a mutant of the type $(4n+1+1-1-1)$. Such forms obviously cannot properly be called $4n$ or tetraploid. They are unbalanced chromosomal mutants of the types indicated in the formulas, and may be classified as modified tetraploids, or at most as "pseudotetraploids."

In previous papers (**4**–**7**) the occurrence of tetraploid plants in *Datura Stramonium* has been announced and their peculiarities briefly discussed. It is the purpose in the present paper to give in some detail the evidence for tetraploidy in this species. This evidence consists in peculiarities in the ratios obtained in the inheritance of simple Mendelian characters, and in the arrangement of the chromosomes which is responsible for these peculiar ratios. Before presenting the data, however, it will be desirable to summarize briefly the work of others on tetraploid plants.

The first tetraploid plant shown to have twice as many chromosomes in its somatic cells as the species from which it had been derived was DEVRIES'S mutant *gigas*, which Miss LUTZ (**18**) and other investigators have shown to have 28 somatic chromosomes instead of the 14 characteristic of the parent species *Oenothera Lamarckiana*. *Gigas* mutants from other species of *Oenothera* have later been discovered, and some of them definitely shown to have the tetraploid number of chromosomes. Thus tetraploidy has been reported in *O. stenomeres* and *O. pratincola* by BARTLETT (**1**, **2**), in *O. grandiflora* by DEVRIES (**28**), and in *O. Lamarckiana* mutant *simplex* by BOEDIJN (**10**). A deVriesian mutant of the *gigas* type which breeds true, and which has been shown to have double the number of chromosomes possessed by the normal parental form, is with little doubt to be considered tetraploid, despite the lack of evidence from the inheritance of Mendelian characters. Breeding data are available, however, in regard to *Oenothera gigas*, as has already been pointed out (**4**), which indicate

that the *nanella* forms produced by *O. gigas* and considered new mutants were in fact recessive segregates from tetraploid parents duplex for the dwarf character.

Plants with the tetraploid number of chromosomes have arisen in the hybrid form *Primula kewensis*, from the cross *P. floribunda*×*P. verticillata*, as well as from the reciprocal cross (DIGBY **11**). The first plant of *P. kewensis* possessed the diploid number of chromosomes but was sterile, having only thrum flowers. Later, however, it produced a pin flower which, when pollinated from a thrum flower, produced seed which gave rise to a race of fertile *P. kewensis* possessing the tetraploid number of chromosomes. FARMER and DIGBY (**12**) showed that the more numerous chromosomes of the fertile form were only half the size of those of the sterile form.

The tetraploid form of *Primula sinensis* is the only case which has been studied heretofore to ascertain the manner of inheritance of Mendelian characters when the chromosomes carrying the factors for these characters have been doubled. GREGORY (**16**), working with this species, discovered tetraploid plants which were heterozygous for certain factors. While some of his plants gave ratios of 3:1 when selfed and 1:1 when back-crossed, other plants gave much higher ratios, which he interpreted as 15:1 for the self and 3:1 for the back-cross. On the basis of these latter ratios, he concluded that there was not independent assortment of the factors involved. The numbers in his pedigrees were rather small to be used to give reliable figures for the higher ratios. MULLER (**22**), however, showed that the figures which GREGORY actually obtained in his higher ratios were somewhat more consistent with a 35:1 ratio for the self and a 5:1 ratio for the back-cross, which would be expected if the chromosomes bearing the factors involved assorted at random in the reduction divisions.

WINKLER (**29**) obtained plants with the tetraploid number of chromosomes in *Solanum nigrum* and in *Lycopersicum esculentum*, after grafting the two species together, and inducing formation of adventitious buds from the callus by cutting across the stem at the place of union of stock and scion. Among a large number of graft chimeras and normal shoots he found three which in appearance

suggested *gigas* forms and which by chromosomal counts were shown to contain tissue with twice the normal number of chromosomes. By this method he secured two plants of the nightshade and one of the tomato which showed the tetraploid number of chromosomes. The former has 72 chromosomes in the diploid stage and the latter has 24. A study of the tissue of diploid tomato plants showed that occasionally cells with a tetraploid number of chromosomes were found in the collenchyma and in the pith. WINKLER suggests that his sprouts with tetraploid chromosome number originated either from tetraploid cells in the tissue which formed the callus, or that a tetraploid condition was brought about in the callus tissue by a union of two diploid nuclei. The work of the MARCHALS (**19**), in which they obtained tetraploid capsules in mosses by inducing sporophytic tissue to grow out into $2n$ gametophytes, the gametes from which united to form $4n$ sporophytes, is an outstanding example of experimental production of tetraploidy.

GATES (**14**) has given in a table of "tetraploid species" a useful list of more or less closely related forms, the chromosomal counts of which differ from each other by multiples of two. TÄCKHOLM (**26**) and HARRISON (**17**), moreover, in their cytological studies on the rose, to mention but a single genus recently investigated, furnish evidence which indicates that duplication of chromosomes may have been of influence in the origin of diverse forms in nature.

Evidence of tetraploidy in Datura

APPEARANCE OF TETRAPLOID PLANTS

Figures of habit and capsules of a tetraploid *Datura* have been given in earlier publications (**5**, **6**). On many-noded tetraploids the leaves are generally broader and larger than those on many-noded diploid plants, and the capsules are more or less globose rather than egg-shaped. The general appearance of such typical tetraploid plants would probably satisfy the student familiar with the *gigas* mutants of *Oenothera*. Few-noded tetraploids, however, are not so distinct in appearance from diploids, and tetraploids of race B, whether many or few-noded, seem largely to lack the broad-leaved lush appearance which we had learned to associate with tetraploid plants of other lines, although they do show the typical spherical

capsule. It seems not unreasonable to attribute such conspicuous peculiarities of tetraploids as the increase in size of leaves, stems, floral parts, and mature seeds to the increase in size of the cells composing these organs. That the size of comparable cells in *Datura* is influenced by the number of chromosomes which they contain, is shown by measurements of pollen grains of tetraploids and diploids, which will be discussed later.

The shape of capsule, spherical instead of ovate, is the outstanding character which gives the appearance of having suffered a qualitative rather than a merely quantitative modification. We believe, however, that the shape of the capsule, although qualitatively different from that of the diploid, in some way is determined by cell size or by altered physiological responses induced by the quantitative change from a $2n$ to a $4n$ condition. A study has been begun upon the changes in external and internal morphology brought about by changes in chromosome number (**24**). It will be advisable, therefore, to defer a more detailed discussion of structural peculiarities to a later publication.

Tetraploids modified more or less conspicuously in appearance of capsule, leaf, and habit by an increase or a decrease in the number of chromosomes away from the balanced $4n$ condition, have already been briefly mentioned (**5**), and will be reserved for detailed discussion in a later publication.

One of the clearest means of distinction between tetraploids and diploids lies in the relative size of their mature pollen grains. Pollen grains of diploids average about 57 μ in diameter, while those of tetraploids average about 68 μ in diameter. Their volumes, therefore, vary approximately as the cubes of their diameters. One would expect such a relation if the size of the cells is proportional to the number of chromosomes which they contain. Pollen of tetraploids is relatively good and shows only slightly more shriveled grains than that of diploids; 3.3 as against 2.7 per cent in a series of counts made in 1920 (**4**).

Distribution of chromosomes in pollen mother cells

In tetraploid mosses it was found by the Marchals (**20**) that the chromosomes at the late prophase and metaphase of the first division of the spore mother cells were mostly in sets of 4. In

tetraploid Daturas the chromosomes at this stage usually form connected sets of 4 chromosomes each. In favorable cases 12 such sets can been seen, and these quadrivalents can be arranged, like their component chromosomes, into the 6 size classes already figured in triploid Daturas (3).

Twenty-seven *Datura* plants, each with a total number of 48 chromosomes, were examined with reference to the distribution of the chromosomes after the first division in the pollen mother cells. Three of these plants showed morphological and cytological peculiarities which led to their being regarded as 48-chromosome plants in which one or more of the 12 sets had 5 chromosomes, and an equal number of sets had 3 chromosomes instead of the normal 4, the total number of chromosomes thus adding up to the $4n$ number 48. Omitting these 3 plants, there were 243 second metaphase plates in which either one or two chromosome plates were counted. Of these, 167 cells showed a distribution of 24–24, and 71 cells showed a distribution of 23–25. Four cells showed 22–26, and one, 21–27.

If the chromosomes are presumed to be all in fours, and double non-disjunction and non-disjunctions of a higher order are temporarily disregarded, then about 30 per cent of cases of simple non-disjunction occur on the average. In 100 pollen grains from tetraploids there would then average about 70 with 24 chromosomes, 15 with 23 chromosomes, and 15 with 25 chromosomes.

If all the 12 kinds of chromosomes undergo non-disjunction equally, then in 100 gametes there will average about 1.25 with only 1 chromosome of a particular set, and 1.25 with 3 chromosomes of this set. In a A_2a_2 plant, for example, in 160 gametes there would be 26 A_2+104 Aa+26 a_2+A+Aa_2+a+A_2a. If we classify the gametes solely as to whether they are dominant or recessive, the formula becomes 133 A+27 a. On selfing, there would be expected 24,871 A to 729 a, or an excess of 18 recessives over the number of recessives in a 35:1 ratio, calculated on the basis of the gametic formula A_2+4Aa+a_2. Hence only about 0.07 per cent of additional recessives would be expected from the gametes resulting from non-disjunction. This would signify an addition of one recessive in a total of 1,422 offspring, an addition which could not be recog-

nized from the breeding data. The back-cross with recessive pollen would give only an addition of one recessive in about 480 offspring. This is based on the extreme assumption that pollen grains and egg cells with unbalanced chromosome numbers are as functional as those with normal constitution. We conclude, therefore, that, notwithstanding the very large amount of non-disjunction in tetraploid Daturas (cf. also GATES (**13**) on tetraploid *Oenothera*), the Mendelian proportions are not sensibly affected.

OCCURRENCE OF NEW TETRAPLOIDS

No. 16336 (9).—The first tetraploid mutant observed in *Datura* was discovered in the summer of 1916 in an F_2 pedigree from the cross of purple, armed by white, *inermis*. The 37 plants in this pedigree showed typical segregation, both for color of flower and for spininess of capsule. The tetraploid mutant no. 16336 (9) turned out to be heterozygous for purple and also for node number, but did not show evidence of being heterozygous for spines. As is our practice in testing new mutants, no. 16336 (9) was used in pollinating triple recessive normals, but although many attempts were made it seemed impossible to obtain crosses between this new mutant and the diploid lines from which it had arisen. Later study seemed to indicate that the new form differed from the other mutants discovered in two important respects, namely, it appeared to breed true, and to be sterile with the form from which it had arisen, although fertile with itself and with its own offspring produced by self-fertilization. For these reasons the mutant race was given the name of "New Species," since it had seemed to satisfy the criteria of a distinct species from its morphological peculiarities, from the fact that it bred true, and especially because it seemed capable of maintaining itself in a mixed population uncontaminated by crossing with its ancestral line. Plant no. 16336 (9) and its offspring are designated race A in our tabulations.

No. 1935 (2).—This tetraploid plant was found in the offspring from the cross between a white, armed, many noded Ilex mutant and a purple, *inermis*, few-noded normal. As was to be expected, the 8 plants in this pedigree showed only the dominant characters, purple, armed, and many nodes, and were presumably heterozygous

for these characters. Plant no. 2, from the shape of its capsules and general appearance, was judged to be a mutant of the "New Species" type. This conclusion seemed confirmed by its ability to take part in crosses with "New Species" plants while being practically sterile with normals. Conclusive evidence of its tetraploid nature was later obtained from tetrasomic ratios in the next generation and chromosomal counts in its offspring. Since it was presumably heterozygous for all the three known Mendelian characters, it was chosen for detailed breeding experiments and formed the origin of tetraploid race B. Its immediate offspring will be discussed later.

No. 1937 (7).—This tetraploid arose in the F_1 from a cross between a white, armed, many-noded Ilex mutant and a purple, *inermis*, many-noded normal, and has much the same origin as 1935 (2). Of the 50 plants in the pedigree, 35 were normals, 14 were Ilex, and 1 (no. 7) was of the "New Species" type and formed the origin of tetraploid race C. It crossed readily with "New Species" plants, but was nearly sterile with normals, and its offspring segregated in typical tetraploid manner. This tetraploid and those previously discussed arose from plants derived from earlier hybridization between line 3[1] which is a white, *inermis*, few-noded form from Germany, and either of two closely related armed, many-noded lines from Washington, D.C., line no. 1 being purple, and line no. 2 being white.

No. 1912 (111).—This tetraploid arose in line no. 10, which is a purple, armed, many-noded form from Virginia which had been selfed for three generations before giving rise to pedigree 1912. The 135 sibs of no. 111 were normal in appearance.

No. 1917 (46).—This tetraploid arose in line no. 18, which is a purple, armed, many-noded form received from Dr. C. M. WOODWORTH under the name of *D. ferox*. It had been selfed for three generations before giving rise to pedigree 1917, which contained 103 normal plants in addition to the tetraploid no. 46.

Nos. 1919 (57), (107).—These tetraploids arose in line no. 19, which is a white, armed, few-noded form received from Dr. C. M.

[1] Line numbers refer to inbred lines, listed in previous publications (54, table VIII, and 60, table II).

WOODWORTH under the name of *D. quercifolia*. It had been selfed for two generations before giving rise to pedigree 1919, which contained 282 normal plants, one possible variant which was not further tested, and two similar plants (nos. 57 and 107) which were described in our notes as rough leaved. No. 107 was not bred from, but was probably the same as no. 57, the offspring of which were shown to be tetraploid. The fact that these plants when first found were not recognized as related to the "New Species" type, is perhaps partially explained by the fact that we had never before obtained a tetraploid in a few-noded race, and in few-noded plants the tetraploid habit appears less distinct than in plants that are many-noded.

In addition to these tetraploids, there occurred also in the summer of 1920, in a pedigree of 70 individuals, three plants which were recorded as similar to the "New Species" in appearance. They were not tested by matings with diploids and tetraploids, but selfed offspring from one of the three showed that this individual was diploid and rendered doubtful the provisional determination of the other two. There remain, therefore, six cases in which tetraploidy is known to have arisen in the cultures, one case (the untested plant in "*D. quercifolia*") which is probably to be considered tetraploidy, and two doubtful cases.

Of the seven probable tetraploid plants discovered, one was found in 1916 and the other six in the summer of 1919. In earlier cultures, before our eyes had become trained in picking out mutants, tetraploids might readily have been overlooked. In later cultures, and especially since 1919, they have been sought with great care, since we wished to obtain them in line no. 1, which has given nearly all of the other mutant types. In the summer of 1919 there were growing in the field about 8,880 plants of diploids or $(2n+1)$ mutants. The six tetraploids from this season give the ratio of about one tetraploid to 1,480 diploids, or modified diploids. In the summer of 1920 we had approximately 14,400, and in the summer of 1921 we had approximately 12,670, $2n$ or $(2n+1)$ plants, to say nothing of a large number of plants grown in the greenhouse each year. From these two later seasons we might have expected about 18.3 tetraploids, if the 1919 records could be considered a random

sample. Why so many appeared in the last mentioned season and not later we can accept merely as a fact without at present being able to offer any explanation of the cause.

ORIGIN OF TETRAPLOIDY

The question naturally arises as to the origin of tetraploidy in these seven plants just discussed. Certain facts about their parentage and their genetic constitution will give a clue as to some of the ways in which the tetraploidy could not have arisen in the specific instances. Thus in two families a tetraploid arose, along with diploids, in the F_1 of a cross between parents (p_2A_2 and P_2a_2) differing in two pairs of genes. The tetraploid was ($P_2p_2A_2a_2$). In these two cases the hypothesis of apogamy of a $4n$ egg cell, advanced by GATES (**14**) for the origin of tetraploidy in *Oenothera*, is excluded.

In one family a tetraploid occurred in the offspring of a selfed diploid plant ($PpAa$) heterozygous for the factors for purple color and for spines. The tetraploid was duplex heterozygous for purple but homozygous for spines, and had the formula ($P_2p_2A_4$). Hence tetraploidy in this case arose after reduction, and the hypotheses of apogamy and of the union of two non-reduced $2n$ gametes are accordingly excluded. The practical impossibility of using $2n$ pollen from a tetraploid in crossing the latter with a diploid appears to be an argument against $2n$ pollen grains assisting in the origin of tetraploids in *Datura*.

The appearance of a relatively large number of tetraploids in a single season suggests the possible influence of some external factor upon the origin of tetraploidy. It is possible that experiments now under way may discover an external stimulus which will induce the production of tetraploids, and enable us to determine the exact stage or stages at which the doubling of the chromosomes may take place.

The spontaneous origin of triploids has not yet been observed in *Datura*. If the frequent triploids in *Oenothera Lamarckiana* and *O. biennis* are produced in the same way as the rare tetraploids in these plants (STOMPS **25**), then the hypothesis of apogamy of a $4n$ egg cell and of doubling of chromosomes in the zygote as an

explanation of tetraploidy, as well as the fertilization of an egg cell by two male nuclei as an explanation of triploidy are inadmissible for these species of *Oenothera*. There remain several possible hypotheses as to the origin of tetraploids in *Datura*, including the union of *2n* gametes which have doubled after reduction and the doubling of chromosome numbers in the zygote. As yet, however, no critical experiments have been made as to the exact time and place of their origin. It is possible, of course, that different tetraploids have originated in different ways.

Self and inter se incompatibilities

It was early noticed that tetraploid capsules contain relatively few seeds, and that there is difficulty in obtaining sets, especially when tetraploids are crossed with other tetraploids. It seemed possible that class differences among the tetraploids might be responsible for the irregular setting of fruit. Accordingly, in the summer of 1917, an experiment was undertaken, the purpose of which was to attempt as many *inter se* crosses as possible among a group of 101 tetraploid plants, as well as crosses between these tetraploids and diploids. The results of the latter part of the experiment are tabulated in table I, and will be discussed in the succeeding section. As to the first part of the experiment, it became evident that, because of the relatively few flowers produced by tetraploid plants, all the possible combinations desired could be made only between a much smaller number of individual plants. Moreover, the irregularities in setting were such that the establishing of incompatibilities between two individuals would have necessitated several attempted crosses between them. Such evidence as was secured, however, gave no support to the idea of *inter se* incompatibilities, and later work, especially the *inter se* crosses between individuals of the pedigrees shown in tables V and VIII, indicated that no scheme of class incompatibilities between pure tetraploids could be derived from the data at hand.

Facts in regard to the setting of seed can be given for the 1917 experiment. A total of 106 attempted selfs are recorded for 68 tetraploids in this series. A total of 90 capsules and 7,445 seeds resulted, giving an average of 70.2 seeds for each self-pollination.

TABLE I

RESULTS OF ATTEMPTED CROSSES BETWEEN TETRAPLOIDS (RACE A) AND DIPLOIDS; SUMMER OF 1917

A:* WHEN DIPLOID IS FEMALE PARENT ($2n \times 4n$)					B:† WHEN TETRAPLOID IS FEMALE PARENT ($4n \times 2n$)							
Females ($2n$)		Number of parents		Polli-nations	Males ($2n$)		Number of parents		Polli-nations	Sets	Seeds	Seedlings
Types	Lines	Female ($2n$)	Male ($4n$)		Types	Lines	Female ($4n$)	Male ($2n$)				
War	2	5	13	24	War	2	46	4	54	22	183	3
War	3	2	3	4	Win	4	19	3	23	11	80	1
Win	4	1	1	1	Pin	1, 4	8	1	8	4	5	0
War	1, 2, 6	3	2	4	Par	1, 4	13	1	17	4	137	0
Pin	1, 4	14	30	45								
Win	1, 4	4	21	21	Totals		86	9	102	41	405	4
Par	1, 4	1	1	1								
Totals		30	71	100								

* A total of 90 different combinations was made; no capsules set.

† A total of 93 different combinations was made; seeds were not classified into large and small; eight seeds from second type (Win) and fifteen from last type (Par) were not planted.

A total of 150 *inter se* crosses were attempted, with a total of 101 capsules with seeds resulting, containing altogether 5,804 seeds, giving an average of 38.7 seeds per attempted cross. The comparable figures for the diploids used at this time in the pollinations shown in table I are: 18 individual selfs attempted, 17 capsules setting containing altogether 6,347 seeds, giving an average of 352.6 seeds per self-pollination; and 10 individuals crossed, 10 capsules setting containing altogether 2,758 seeds, giving an average of 275.8 seeds per attempted cross. The difference between the amount of seed produced by tetraploids and by diploids is strikingly evident from these figures. In both cases the heavier set of seed when a flower is selfed is probably due, in large measure at least, to the larger amount of pollen usually available for growth on the stigma, rather than to incompatibilities between individuals.

Crossability of tetraploids and diploids

The difficulty of making crosses between the "New Species" and the normal *Datura* from which it had arisen was early recognized as a peculiarity of the mutant which we have since learned to know as tetraploid. The likelihood of obtaining offspring from attempted crosses between a tetraploid and a diploid depends upon which way the pollination is made. If the $4n$ plant is used as the female and the $2n$ plant as the male, an occasional seed may be expected; if the $2n$ plant is the female and the $4n$ the male, failure to set seed is practically invariable. A complete record has not been kept of all the pollinations which failed to set seed, although successful sets have been recorded. In three seasons, however, tabulations are possible for both sets and failures.

Diploids × tetraploids.—The combination ($2n \times 4n$) with the diploid used as female may be considered first. In the summer of 1917 a total of 100 pollinations, representing 90 different combinations, were made between diploids and tetraploids, as shown in table IA. These 100 pollinations of the type ($2n \times 4n$) were complete failures. The same type of cross was attempted in the summer of 1919. Using tetraploids of the same race A, a total of 88 pollinations were made, but again without effecting the production of seed. In the summer of 1921, 24 pollinations of diploids

were made with pollen from tetraploids of race B, and again no capsules set. We have therefore in three systematic attempts to obtain seed by crossing diploids by tetraploids a total of 212 failures and no successes. To these figures might be added other attempts of which detailed records of failures were not made. Thus in the summer of 1920, a considerable number of pollinations were made of diploid females by tetraploid males. The records for 1920 show there set only 2 capsules, containing a total of 27 small seeds, none of which germinated. There are four instances, however, where the cross ($2n \times 4n$) seemed to give viable seed, but in three of the four cases the results might have come from accidental selfing of the female parent, and the fourth case is more likely due to an experimental error than to the cross attempted.[2] It will be seen, therefore, that there are no unquestionable data against and many in favor of the conclusion that in *Datura* the cross is incompatible between diploids and tetraploids when the diploid is the female parent.

TETRAPLOIDS × DIPLOIDS.—The combination ($4n \times 2n$) with the tetraploid used as the female parent differs from the reciprocal combination in that it occasionally leads to viable offspring. In the summer of 1917, 102 crosses were attempted, using pollen from diploids on tetraploid females, and a total of 405 seeds were obtained which were not classified as to size. These seeds were all planted except a total of 23 from two of the capsules, and produced 4 offspring, as shown in table I*B*. Through a misunderstanding

[2] Of the three cases in the greenhouse in 1916–17, two were pollinations of white, *inermis* diploids by pollen from purple, armed tetraploids, and gave rise to 2 and 23 seedlings respectively, all of which were identical in appearance, as also in the offspring which they later produced, to the female diploid parents. Since the tetraploid line A is not known to contain factors for the *inermis* character, it is difficult to account for the results by crossing. The other pollination, a purple, armed diploid by a purple, armed tetraploid, gave rise to a single purple, armed plant, diploid in appearance, but since unfortunately it was not bred from, it cannot with certainty be classified either as a successful cross or an error in technique. The fourth doubtful case was one out of four attempted crosses between a homozygous purple, armed diploid and the tetraploid of line B, which was duplex for the three characters, purple color, armed capsules, and many nodes. The single offspring was a white, armed, many-noded plant, proved to be diploid by chromosomal counts, and could not have come from selfing. It is unlikely that it came from the cross attempted and may most wisely be attributed to some experimental error, such as an accidental admixture of seeds or labels.

these 4 seedlings were discarded at an early stage, and no data is available from the breeding behavior in regard to their chromosomal constitution.

In the summers of 1920 and 1921 systematic attempts were again made to obtain crosses, and the seeds were classified as to size, and the chromosomal constitution of the offspring was determined. The results are tabulated in tables II and III. The seeds listed as large for the most part were normal in appearance, although often somewhat reduced in size, while those labeled small were distinctly smaller and in most experiments would not be considered worth

TABLE II

RESULTS OF POLLINATING TETRAPLOIDS DERIVED FROM LINE 10 BY A DIPLOID 20509(5) FROM LINE 10 ($4n \times 2n$); SUMMER OF 1921

$4n$ SEED PARENT	POLLINATIONS	CAPSULES	SEEDS		SEEDLINGS	SEEDLINGS PER POLLINATION	TYPES OF SEEDLINGS				
			Large	Small			$2n$	$2n+1$	$3n-1$	$3n$	$4n$
20563(2)	1	1	2	0	0	0.00	....	....	....	....	....
(3)	1	1	3	0	0	0.00	....	....	....	....	....
(4)	20	17*	62	3	6	0.30	2	1	1	1	1
(5)	7	6*	13	1	0	0.00	....	....	....	....	....
(6)	8	7*	26	1	4†	0.50	3	....	....	....	....
(7)	6	6	31	3	4†	0.67	2	....	....	1	....
(8)	5	0	0	0	0	0.00	....	....	....	....	....
(9)	5	5	20	2	2	0.40	1	....	....	1	....
(10)	9	0	0	0	0	0.00	....	....	....	....	....
Totals	62	43	157	10	16	0.26	8	1	1	3	1

* One of the capsules which set contained no seeds.
† One seedling died without chromosomal count.

planting. A sharp classification, however, between the large and small is not always possible. Since, however, small seeds in rare instances have been found to germinate, they have been listed separately in the table and planted with the others. A difference seems to exist between line 10 and line 18 in regard to the proportion of small to large seeds and in regard to the mean number of viable seeds which they produce per pollination. It will not be desirable to present data of isolated attempts at crossing tetraploids with diploids. Enough has been given to show, in several rather extensive tests where tetraploids were pollinated by normals, that a few viable seeds have been obtained and that the offspring of such a

TABLE III

SUMMARY OF RESULTS OF POLLINATING TETRAPLOIDS BY DIPLOIDS ($4n \times 2n$); SUMMERS OF 1920 AND 1921

YEAR	$4n$ RACE	$2n$ RACE FROM LINE	NO. $4n$ SEED PARENTS	POLLINATIONS	CAPSULES	SEEDS		SEEDLINGS	SEEDLINGS PER POLLINATION	TYPES OF SEEDLINGS					
						Large	Small			$2n$	$2n+1$	$3n-1$	$3n$	$3n+1$	$4n$
1920	A	1, 3, 4	3	60	33	211	2236	3	0.05	1	1	....	1	....	....
1920	B	4	20	102	34	69	32	4†	0.04	1	....	....	2	....	....
1921	Line 10	10	9	62	43	157	10	10††	0.26	8	1	1	3	....	1
1921	Line 18	18	8*	54*	22	45	1165	4	.....	2	....	....	1	1	....
Totals	.....	.....	40	278*	132	482	3443	27	.....	12	2	1	7	1	1

* The three tetraploid plants which produced offspring had other infertile pollinations which were not recorded.

† One seedling died without chromosome count.

†† Two seedlings died without chromosome count.

cross contain both diploids ($2n$) and triploids ($3n$). Unfortunately it cannot be proved that the single tetraploid seedling from the pollination of a tetraploid by a diploid was not due to accidental selfing of the female $4n$ parent, since both pollen and seed parents from which it arose were from the same inbred line. Further extensive tests involving the proper combinations of Mendelian factors are being undertaken, and until there are more data on the subject, it does not appear wise to discuss in detail the types of offspring obtained from the cross between tetraploids and diploids.

Tetrasomic inheritance

Formulas and ratios.—Since in somatic cells of a tetraploid *Datura* each of the 12 chromosomal sets consists of 4 homologous chromosomes instead of only 2 as in diploids, it is obvious that in dealing with the transmission of Mendelian characters we have to do with tetrasomic rather than with the disomic inheritance more familiar to students of heredity. The convenient terms nulliplex, simplex, duplex, triplex, and quadriplex, can be used in regard to the number of chromosomes containing a given gene, without thereby admitting the amount of so-called dominance of the factors in question. In diploids there is only one type of heterozygote, and this may be written Aa. In tetraploids, however, there may be three types: simplex ($Aaaa$), duplex ($AAaa$), and triplex ($AAAa$). If we start with a tetraploid plant duplex for the factor A, and number each individual chromosome by a Roman superscript, we have the formula $A^{I}A^{II}a^{III}a^{IV}$. If at tetrasomic disjunction the chromosomes $A^{I}A^{II}$ go to one pole, then the chromosomes $a^{III}a^{IV}$ may be expected to go to the other pole of the dividing germ nucleus. If the assortment of chromosomes takes place at random, as the evidence seems to indicate is the case, there should be all the possible paired combinations of the different chromosomes in equal numbers, and the gametes consequently should be: $A^{I}A^{II}+a^{III}a^{IV}$, $A^{I}a^{III}+A^{II}a^{IV}$, $A^{I}a^{IV}+A^{II}a^{III}$. In summing the combinations we may drop the superscripts which refer to individual chromosomes, and consider merely the different combinations of the allelomorphic pair A and a. The gametic formula therefore becomes $AA+4\,Aa+aa$. Because of the large number of chromo-

somes to be taken into account (regularly 4 in pure tetraploids and as many as 7 in $(4n+3)$ mutants), it has been found convenient to make use of subscripts and to write the gametic formula $A_2+4\ Aa+a_2$. Selfing such a duplex plant should give the following genetic types of zygotes: $1\ A_4+8A_3a+18\ A_2a_2+8\ Aa_3+1\ a_4$. The phenotypic ratio therefore is $35\ A:1\ a$. An inspection of the gametic ratio shows that back-crossing a duplex plant (A_2a_2) with a homozygous recessive with the formula a_4 should give the following genetic types: $1\ A_2a_2+4\ Aa_3+1\ a_4$, or a phenotypic ratio of $5\ A:1\ a$.

Table IV shows the various formulas and the calculated ratios when a single pair of factors are involved in tetrasomic inheritance. Instead of only two phenotypic ratios obtainable from heterozygous diploids (3:1 and 1:1), it will be seen there are five phenotypic ratios possible for heterozygous tetraploids, namely, 35:1, 11:1, 5:1, 3:1, and 1:1. These ratios have been calculated from the results of a random assortment of the chromosomes. They are supported, moreover, by the breeding data in reference to two pairs of Mendelizing characters, which will be presented in the following section.

BREEDING DATA.—Not only do the capsules of tetraploids contain a smaller number of seeds than those of diploids, but the seeds which they contain show a distinctly lower percentage of germination. Due to this reduction in viable seed, the cultures of race A in many cases are too small to give reliable evidence as to the genetic composition of their parents. In studying race B an especial effort was made to insure an adequate number of plants in the various progeny cultures, so that no reasonable doubt need exist, we believe, as to the proper classification of the parents involved. Accordingly, the data in regard to race B will be presented first, together with the two cultures of race C, since these contained sufficient numbers of individuals to give reliable evidence as to the ratios involved.

It will be remembered that the first plant of race B was in an F_1 from a cross between a triple recessive and a triple dominant, and, as the breeding data later showed, was duplex heterozygous for the three characters, color, spines, and nodes. The parent of race C was duplex for color and spines but not for nodes, and its offspring conse-

quently showed segregation only for the first two characters. The node number characters were found difficult to classify in recording tetraploid plants, partially perhaps on account of the unfavorable environmental conditions under which the tetraploid cultures showing segregation for node number were grown, and possibly also because these characters may be less distinct in tetraploids

TABLE IV

TETRASOMIC INHERITANCE OF AN ALLELOMORPHIC PAIR (A, a) IN TETRAPLOID DATURAS; FORMULAS FOR PARENTS, GAMETES, AND OFFSPRING

PARENTS (GAMETES IN PARENTHESES)	MATED WITH	GENETIC TYPES OF OFFSPRING					RATIO A:a
		A_4	A_3a	A_2a_2	Aa_3	a_4	
A_4 (A_2)	A_4	1	.....	.....	.....	.....	∞ :0
	A_3a	1	1	.....	.....	.....	∞ :0
	A_2a_2	1	4	1	.....	.....	∞ :0
	Aa_3	.....	1	1	.....	.....	∞ :0
	a_4	.....	.....	1	.....	.....	∞ :0
A_3a (A_2+Aa)	A_4	1	1	.....	.....	.....	∞ :0
	A_3a	1	2	1	.....	.....	∞ :0
	A_2a_2	1	5	5	1	.....	∞ :0
	Aa_3	.....	1	2	1	.....	∞ :0
	a_4	.....	.....	1	1	.....	∞ :0
A_2a_2 (A_2+4 Aa+a_2)	A_4	1	4	1	.....	.....	∞ :0
	A_3a	1	5	5	1	.....	∞ :0
	A_2a_2	1	8	18	8	1	35:1
	Aa_3	.....	1	5	5	1	11:1
	a_4	.....	.....	1	4	1	5:1
Aa_3 (Aa+a_2)	A_4	.....	1	1	.....	.....	∞ :0
	A_3a	.....	1	2	1	.....	∞ :0
	A_2a_2	.....	1	5	5	1	11:1
	Aa_3	.....	.....	1	2	1	3:1
	a_4	.....	.....	.....	1	1	1:1
a_4 (a_2)	A_4	.....	.....	1	.....	.....	∞ :0
	A_3a	.....	.....	1	1	.....	∞ :0
	A_2a_2	.....	.....	1	4	1	5:1
	Aa_3	.....	.....	.....	1	1	1:1
	a_4	.....	.....	.....	.....	1	0:∞

than in diploids. It seems best, therefore, to present only the data upon the two characters, color and spininess, which can be classified without question, despite the fact that the records for node character are in conformity with the data which are presented here, if allowance is made for a rather wide range of errors in classification.

The breeding data for races B and C are summarized in tables V and VIII. The first tetraploids in these races are listed as B

TABLE V

TETRASOMIC INHERITANCE OF ALLELOMORPHIC CHARACTERS PURPLE (P) AND WHITE (p) FLOWER COLOR IN TETRAPLOID RACES B AND C; LETTERS B AND C ALONE REPRESENT PARENT PLANTS OF THESE TWO RACES; LETTER B FOLLOWED BY A NUMERAL REPRESENTS INDIVIDUAL PLANT IN OFFSPRING OBTAINED BY SELFING PLANT B; LETTER A REPRESENTS PLANTS OF TETRAPLOID RACE A

a: PARENTS QUADRIPLEX (P_4) OR TRIPLEX (P_3p) FOR PURPLE FACTOR

P_4 or $P_3p \times P_4$ or P_3p			P_4 or $P_3p \times p_4$ and reciprocal			P_4 or $P_3p \times Pp_3$ and reciprocal			P_4 or $P_3p \times P_2p_2$ and reciprocal		
Theoretical ratio	∞	0	Theoretical ratio	∞	0	Theoretical ratio	∞	0	Theoretical ratio	∞	0
Pollination	P	p	Pollination	P	p	Pollination	P	p	Pollination	P	p
B(5) self.....	326	0	B(5)×B(2).....	90	0	B(5)×B(11).....	75	0			
B(14) self.....	127	0	B(14)×B(2).....	85	0						
			B(14)×B(3).....	77	0						
B(18) self.....	366	0	B(18)×B(2).....	136	0	.....	.....	.....	B(18)×B(10)....	49	0
B(22) self.....	264	0	B(22)×B(2).....	67	0				B(22)×B(10)....	35	0
B(26) self.....	118	0	B(26)×B(2).....	77	0				B(26)×B(10)....	44	0
Totals.....	1201	0	Totals.....	532	0	Totals.....	75	0	Totals.....	128	0
Calculated.....	1201	0	Calculated.....	532	0	Calculated.....	75	0	Calculated.....	128	0

b: PARENTS DUPLEX (P_2p_2) FOR PURPLE FACTOR

$P_1p_1 \times P_2p_2$			$P_1p_1 \times p_1$ and reciprocal			$P_1p_1 \times P_3p_3$ and reciprocal			$P_1p_1 \times P_3p$ or P_4 and reciprocal		
Theoretical ratio	35	1	Theoretical ratio	5	1	Theoretical ratio	11	1	Theoretical ratio	∞	0
Pollination	*P*	*p*	Pollination	*P*	*P*	Pollination	*P*	*p*	Pollination	*P*	*p*
B self	232	10	A×B	8	4						
C self	393	12	A×C	25	7						
B(4) self	273	10	B(4)×B(2)	86	17						
B(4)×B(10)	70	0									
B(7) self	292	11	B(7)×B(2)	146	22	B(7)×B(11)	24	3			
B(7)×B(10)	63	2	B(7)×B(3)	100	24	B(7)×B(8)	22	6			
						B(7)×B(13)	45	5			
						B(13)×B(7)	14	1			
						B(17)×B(7)	13	0			
						B(6)×B(7)	36	3			
B(9) self	555	18	B(9)×B(2)	148	18	B(13)×B(9)	51	6			
B(9)×B(10)	130	2									
B(10) self	89	2	B(10)×B(2)	5	3	B(13)×B(10)	136	11	B(18)×B(10)	49	0
			B(10)×B(3)	18	5	B(25)×B(10)	53	7	B(22)×B(10)	35	0
			B(2)×B(10)	58	14				B(26)×B(10)	44	0
			B(3)×B(10)	81	13						
B(19) self	174	6	B(19)×B(2)	41	12						
B(20) self	220	5	B(20)×B(3)	38	10						
B(21) self	321	16	B(21)×B(2)	98	20						
B(21)×B(10)	53	1									
B(29) self	253	8	B(29)×B(2)	53	10						
B(29)×B(10)	107	3									
Totals	3225	106	Totals	905	179	Totals	394	42	Totals	128	0
Calculated	3238.5	92.5	Calculated	903.3	180.7	Calculated	399.6	36.3	Calculated	128	0
Dev./P.E. $= \frac{13.5}{6.39} = 2.113$			Dev./P.E. $= \frac{1.7}{8.37} = 0.206$			Dev./P.E. $= \frac{5.7}{3.89} = 1.465$					

TABLE V—*Continued*

c: PARENTS SIMPLEX (Pp_3) FOR PURPLE FACTOR

$Pp_3 \times Pp_3$			$Pp_3 \times p_4$ and reciprocal			$Pp_3 \times P_4p_3$ and reciprocal			$Pp_3 \times P_3p$ or P_4 and reciprocal		
Theoretical ratio	3	1	Theoretical ratio	1	1	Theoretical ratio	11	1	Theoretical ratio	∞	0
Pollination	*P*	*p*	Pollination	*P*	*p*	Pollination	*P*	*p*	Pollination	*P*	*p*
B(6) self	218	64				B(6)×B(7)	36	3			
B(8) self	208	69				B(7)×B(8)	22	6			
B(11) self	166	67	B(11)×B(2)	9	10	B(7)×B(11)	24	3	B(5)×B(11)	75	0
			B(11)×B(3)	19	18						
			B(2)×B(11)	9	10						
			B(3)×B(11)	12	10						
B(13) self	435	157	B(13)×B(2)	18	17	B(13)×B(7)	14	1			
			B(13)×B(3)	9	14	B(13)×B(10)	136	11			
						B(13)×B(9)	51	6			
						B(7)×B(13)	45	5			
B(17) self	262	75	B(17)×B(2)	46	53	B(17)×B(7)	13	0			
B(25) self	235	87	B(25)×B(2)	54	39	B(25)×B(10)	53	7			
B(27) self	70	26	B(27)×B(2)	17	12						
B(27)×B(11)	34	9	B(27)×B(3)	21	16						
B(30) self	185	61	B(30)×B(3)	78	67						
B(30)×B(11)	0	1									
B(31) self	70	26									
Totals	1883	642	Totals	292	266	Totals	394	42	Totals	75	0
Calculated	1893.7	631.3	Calculated	279	279	Calculated	399.6	36.3	Calculated	75	0
$\frac{\text{Dev.}}{\text{P.E.}} = \frac{10.7}{14.68} = 0.729$			$\frac{\text{Dev.}}{\text{P.E.}} = \frac{13.00}{7.97} = 1.631$			$\frac{\text{Dev.}}{\text{P.E.}} = \frac{5.7}{3.89} = 1.465$					

d: PARENTS NULLIPLEX (p_4) FOR PURPLE FACTOR

$p_4 \times p_4$			$p_4 \times Pp_3$ and reciprocal			$p_4 \times P_2p_3$ and reciprocal			$p_4 \times P_3p$ or P_4 and reciprocal		
Theoretical ratio	0	∞	Theoretical ratio	1	1	Theoretical ratio	5	1	Theoretical ratio	∞	0
Pollination	*P*	*p*	Pollination	*P*	*p*	Pollination	*P*	*p*	Pollination	*P*	*p*
						A×B.....	8	4			
						A×C.....	25	7			
B(2) self.....	0	303	B(2)×B(11).....	9	10	B(2)×B(10).....	58	14	B(5)×B(2).....	90	0
			B(11)×B(2).....	9	10	B(4)×B(2).....	86	17	B(14)×B(2).....	85	0
			B(13)×B(2).....	18	17	B(7)×B(2).....	146	22	B(18)×B(2).....	136	0
			B(17)×B(2).....	46	53	B(9)×B(2).....	148	18	B(22)×B(2).....	67	0
			B(25)×B(2).....	54	39	B(10)×B(2).....	5	3	B(26)×B(2).....	77	0
			B(27)×B(2).....	17	12	B(19)×B(2).....	41	12			
						B(21)×B(2).....	98	20			
						B(29)×B(2).....	53	10			
B(3) self.....	0	260	B(3)×B(11).....	12	10	B(3)×B(10).....	81	13	B(14)×B(3).....	77	0
			B(11)×B(3).....	19	18						
			B(13)×B(3).....	9	14	B(7)×B(3).....	100	24			
			B(27)×B(3).....	21	16	B(10)×B(3).....	18	5			
			B(30)×B(3).....	78	67	B(20)×B(3).....	38	10			
Totals.....	0	563	Totals.....	292	266	Totals.....	905	179	Totals.....	532	0
Calculated.....	0	563	Calculated.....	279	279	Calculated.....	903.3	180.7	Calculated.....	532	0
			$\frac{\text{Dev.}}{\text{P.E.}} = \frac{13.00}{7.97} = 1.631$			$\frac{\text{Dev.}}{\text{P.E.}} = \frac{1.7}{8.27} = 0.206$					

and C respectively. B(2), B(3), etc., are individual F_1 plants obtained by selfing B. In the summer of 1920, some 30 of the F_1 individuals from race B were planted out with increased space for development, with the hope that it might be possible to analyze with some degree of thoroughness so small an F_1 population by selfing, back-crossing, and by other matings. Plants B(2) and B(3) served as recessives for the purple-white character pair, and plants B(10) and B(11) as recessives for the armed-*inermis* pair. Owing to lack of sufficient seed secured from certain combinations, or to other causes, the number of individuals from this group that were finally used as parents was reduced to 24.[3]

Inheritance of purple pigmentation.—Races B and C.—The data for the inheritance of purple pigmentation in races B and C are shown in table V. The various parents are grouped in sections *a* to *d* according to their genetic constitution, as indicated by the phenotypic ratios shown in their offspring.

The genetic ratios expected from random assortment are given in table IV, from which it is evident that parents quadriplex (P_4) or triplex (P_3p) for the purple factor should show only purples in their immediate offspring, whether they are selfed, or crossed with duplex (P_2p_2), with simplex (Pp_3), or even with nulliplex (p_4) parents. Quadriplex (P_4) and triplex (P_3p) parents are accordingly combined in section *a*. Their separation could be made by breeding a further generation, as has been done in race A, to be considered later. The number of offspring obtained by selfing B(14) and B(26) are not large if the progeny cultures alone were used to distinguish a genetic constitution which should give only purples from a duplex constitution (P_2p_2) which should give a 35:1 ratio of purples to whites. That these as well as the other parents listed

[3] Parent B(7) was a $(4n+1)$ Globe, but since the extra chromosome was neither in the set which carries the genes for purple and white, nor in the set which carries those for armed and *inermis*, it can be classed with the other tetraploids so far as the inheritance of these characters is concerned. Parent B(24) was omitted from the tables, since the ratios given by its offspring, both as to color and spines, showed a wide departure from those given by other parents in race B, and suggest the influence of some unknown factor. Its offspring from selfing, classified as to color and spines, showed 414 purple to 39 white individuals, and 415 armed to 38 *inermis* individuals. The ratio for both the two pairs of characters was thus approximately 11:1 instead of 35:1 expected of duplex parents. The matter is receiving further investigation.

in section *a* are not duplex for the purple factor is clearly shown by the back-crosses to the recessive which give no whites.

Ten duplex (P_2p_2) parents are listed in section *b* with a total of 3,331 offspring from selfing or crossing with a parent of similar constitution, of which 3,225 were purple and 106 white. This makes a close approach to the 35:1 ratio expected. It is realized that the accuracy of the probable error as computed by the conventional method decreases as one departs from a 1:1 ratio, and in consequence that its significance is relatively slight for a 35:1 ratio. The fact, however, that the deviation of the observed from the calculated ratio is only about twice the probable error is an argument so far as it goes that the two ratios are alike. Inspection alone would show that the observed proportions could not agree as well with any other ratio known for disomic, trisomic, or tetrasomic inheritance. The same duplex parents which, when selfed, give a 35:1 ratio give a 5:1 ratio when back-crossed to the recessive (p_4). In a total of 1,084 individuals from such a cross the deviation from a 5:1 ratio is less than the probable error. The duplex (P_2p_2) parent crossed with a simplex (Pp_3) parent gave in a total of 436 individuals a ratio of purples to whites, the deviation of which from the expected 11:1 ratio was less than 1.5 times the probable error.

Nine simplex (Pp_3) parents are listed in section *c*. Out of 2,525 offspring from selfing or crossing with parents of like constitution a proportion of purples to whites was obtained, the deviation of which from a 3:1 ratio was less than the probable error. The back-cross to the recessive (p_4) produced 558 offspring, in which the deviation of the purples and whites from a 1:1 ratio was only 1.63 times the probable error.

Section *d* shows that the two white parents bred true for the recessive character (p_4), and repeats the tabulations already discussed under the previous sections.

It is evident from the data presented in table V that the inheritance of purple pigmentation in the tetraploids investigated is in accord with the ratios obtained in table IV, on the assumption of a random assortment of the chromosomes which contain the genes involved.

Race A.—As previously explained, the first plant of race A appeared in an F_2 from a cross between a purple, armed and a white, *inermis* plant. It was duplex heterozygous (P_2p_2) for color but undoubtedly homozygous for spines, since otherwise *inermis* individuals should have appeared among some of its immediate offspring or its later descendants. Unfortunately the individual parents in race A are not as adequately analyzed by back-crosses and by large numbers of offspring from selfing as are those of race B. Two corrections, therefore, have been found desirable. First is the elimination of all cultures containing less than 20 individuals, except as a self or a back-cross from the same parent is an addition to a culture with 20 individuals.[4] Second is a preliminary classification in table VI of all progeny from selfing which had only purple individuals, according to probabilities of their actually representing 35:1 ratios. The probability values are taken from recently published tables (**9**). The discarding of all pedigrees with less than 20 individuals practically eliminates the possibility of any of the all dominant pedigrees representing a 3:1 or a 1:1 ratio, since, as the tables show, such a pedigree of 20 individuals has only three chances in 1,000 of representing a 3:1 ratio. The pedigrees in table VI therefore represent P_4 or P_3p parents on the one hand, which should give only purple offspring whether selfed or back-crossed, or P_2p_2 parents on the other hand, which should produce 35:1 ratios from selfing and 5:1 ratios from back-crossing to the recessive. The chances of the cultures coming from P_2p_2 parents is represented under the heading "probability." Under *a* are listed the cultures with a probability greater than 0.01, and under *b* those with a probability less than 0.01. Cultures which have less than one chance in 100 of representing a 35:1 ratio can reasonably be classified as coming from quadriplex (P_4) or triplex (P_3p) parents which may be expected to produce only purple offspring. Those in table VI*a* show greater chances of representing 35:1 ratios, and undoubtedly some of them should be classified under duplex (P_2p_2) parents. Without their inclusion in table VII*b*, we have from selfing duplex (P_2p_2) parents 6,595 purples to 225 whites, which gives too few pur-

[4] In addition, one parent was omitted which gave 4 purples to 1 white when selfed, and 20 purples to 9 whites when back-crossed to the recessive. The parent was evidently either duplex or simplex, but from the figures it is impossible to decide which.

TABLE VI

PARENTS IN TETRAPLOID RACE A WHICH PRODUCED ONLY PURPLE (P) OFFSPRING WHEN SELFED OR BACK-CROSSED TO RECESSIVE (p_4); FIGURES UNDER "PROBABILITY" DENOTE PROBABILITY THAT CULTURES OF SIZE OF THOSE GROWN WOULD CONTAIN NO RECESSIVE INDIVIDUALS IF SEEDS PLANTED REALLY REPRESENTED A 35:1 RATIO; PARENTS KNOWN TO BE TRIPLEX (P_3p) FOR PURPLE ARE REPRESENTED IN TABLE VII*a*

a: CULTURES WITH PROBABILITY GREATER THAN 0.01

SELF					BACK-CROSS × p_4				
Pedigree Number	Parent	P	p	Probability	Pedigree Number	Parents	P	p	Probability
17128.....	16848(12)	22	0	0.538					
19746.....	19378(3)	25	0	0.494					
17126.....	16848(11)	35	0	0.373					
20228.....	19781(12)	39	0	0.333					
20221.....	19743(20)	41	0	0.315					
17440.....	17140(18)	46	0	0.274					
19752.....	19389(4)	51	0	0.238					
20219.....	19743(18)	53	0	0.225					
19744.....	19378(1)	65	0	0.160					
20232.....	19781(30)	70	0	0.139					
19750.....	19389(2)	71	0	0.135					
19790.....	19116(1)	72	0	0.132					
17108.....	16848(4)	74	0	0.124	17492.....	16848(4)×17140(37)	7	0	0.279
20231.....	19781(28)	74	0	0.124					
20233.....	19783(15)	78	0	0.111					
20225.....	19781(3)	80	0	0.105					
19749.....	19389(1)	81	0	0.102					
19378.....	19102(12)	84	0	0.094	19379.....	19102(12)×1986(2)	14	0	0.078
19771.....	19383(2)	86	0	0.089					
20214.....	19743(12)	86	0	0.089					
19788.....	19413(4)	90	0	0.079					
17119.....	16848(9)	101	0	0.058	17122.....	16848(9)×16775(3)	8	0	0.233
					17121.....	16079(7)×16848(9)	2	0	0.694
19782.....	19409(3)	106	0	0.051					
19101.....	17123(3)	115	0	0.039					
19781.....	19409(2)	115	0	0.039					
20224.....	19743(23)	121	0	0.033					
19778.....	19302(4)	137	0	0.021					
20226.....	19781(5)	137	0	0.021					
20229.....	19781(17)	141	0	0.019					
20222.....	19743(21)	148	0	0.016					
19753.....	19389(5)	160	0	0.011					
Total...	.....	2604	0	.....	Total..	.....	31	0	.....

b: CULTURES WITH PROBABILITY LESS THAN 0.01

19111.....	17304(1)	165	0	0.010	19112....	17391(1)×17304(6)	3	0	0.579
					19113....	17394(1)×17438(3)	30	0	0.004
20207.....	19743(6)	209	0	0.003					
20218.....	19743(16)	210	0	0.003					
20223.....	19743(22)	250	0	0.001					
20212.....	19743(11)	269	0	<0.001	20213....	19743(11)×19728(3)	49	0	<0.001
20216.....	19743(14)	285	0	<0.001					
20206.....	19743(5)	289	0	<0.001					
20215.....	19743(13)	304	0	<0.001					
20203.....	19743(3)	340	0	<0.001	20204....	19743(3)×19728(3)	26	0	0.009
20211.....	19743(10)	345	0	<0.001					
20205.....	19743(1)	376	0	<0.001					
20227.....	19781(7)	461	0	<0.001					
Totals..	.....	3503	0	.....	Totals..	.....	108	0	.....

TABLE VII

TETRASOMIC INHERITANCE OF ALLELOMORPHIC CHARACTERS PURPLE (P) AND WHITE (p) FLOWER COLOR IN TETRAPLOID RACE A

b: PARENTS DUPLEX (P_2p_2) FOR PURPLE FACTOR; PARENTS MARKED WITH ASTERISK (*) WERE FROM PEDIGREES WHICH HAD SHOWN A 3:1 RATIO FOR PURPLE AND WHITE

$P_2p_2 \times P_4p_2$				$P_4p_4 \times p_4$ and reciprocal			
Theoretical ratio		35	1	Theoretical ratio		5	1
Pedigree Number	Parent	P	p	Pedigree Number	Parent	P	p
16848.....	16336(9) self	134	10	17482.....	16336(9)×17135(13)	14	3
17139.....	16336(9) self	8	0	17446.....	16336(9)×17103(5)	29	6
17140.....	16336(9) self	56	3				
17304.....	16848(7) self	44	2	17449.....	16848(7)×17103(5)	12	3
17123.....	16848(10) self	66	2	17474.....	16848(10)×17106(1)	21	3
				17493.....	16848(10)×17140(37)	9	3
17395.....	16848(16) self	116	4	17475.....	16848(16)×17106(1)	3	1
17441.....	17140(50) self	77	3				
17442.....	17140(55) self	132	6				
17421.....	17106(6)* self	46	1	17501.....	17106(6)×17140(37)	1	3
				17505.....	17106(8)×17140(37)	57	13
17427.....	17106(17)* self	113	6				
17431.....	17110(8) self	66	3				
17430.....	17135(14)* self	37	4				
19102.....	17123(2) self	121	5	19105.....	17123(2)×17438(3)	61	14
19108.....	17394(3) self	173	7	19110.....	17394(3)×17438(3)	12	3
19114.....	17427(10)* self	123	5	19115.....	17427(10)×17438(3)	2	0
19354.....	1996(5)* self	58	1	19355.....	1996(5)×1986(1)	4	0
				19357.....	1996(5)×1989(6)	8	2
				19358.....	1989(6)×1996(5)	4	1
19359.....	1996(6)* self	126	6	19360.....	1996(6)×1986(1)	8	4
				19361.....	1986(1)×1996(6)	12	0
19373.....	19102(3) self	287	12	19374.....	1986(10)×19102(3)	2	3
				19375.....	1989(17)×19102(3)	10	5
19377.....	19102(7) self	42	1				
19380.....	19102(13) self	115	1	19381.....	19102(13)×1986(2)	13	0
				19382.....	19102(13)×1989(3)	6	0
19383.....	19102(14) self	57	1				
19392.....	19108(7) self	210	7	19393.....	19108(7)×1986(5)	3	2
				19394.....	1986(5)×19108(7)	24	8
				19395.....	19108(7)×1989(10)	24	5
19409.....	19108(12) self	184	3	19410.....	19108(12)×1989(19)	5	2
				19411.....	1989(19)×19108(12)	63	9
19413.....	19108(16) self	702	21	19414.....	19108(16)×1989(12)	71	13
				19415.....	1989(12)×19108(16)	37	10
19416.....	19110(2) self	441	14				
19751.....	19389(3) self	82	4	Totals.....		515	122
19754.....	19354(1) self	66	1	Calculated.....		530.8	106.2
19759.....	19359(2) self	144	4				
19780.....	19109(1) self	141	0				
19783.....	19109(4) self	110	4	Dev. $= \frac{15.8}{6.34} = 2.492$			
19786.....	19413(2) self	171	3	P.E.			
19792.....	19416(3) self	35	2				
19796.....	19350(3)* self	43	1				
19807.....	19363(1)* self	69	0	Totals of tables VI*a* and VII*b*...		546	122
19809.....	19367(1)* self	104	3	Calculated.....		556.7	111.3
19812.....	19367(4)* self	134	5				
19815.....	19370(2)* self	93	4	Dev. $= \frac{10.7}{6.49} = 1.649$			
19820.....	19371(2)* self	84	3	P.E.			
19845.....	19396(3)* self	125	1				
19851.....	19399(4)* self	155	7				
19862.....	19405(5)* self	113	3				
19865.....	19412(3)* self	135	2				
19869.....	19417(2)* self	170	8				
20201.....	19743(1) self	106	2				

TABLE VII—*Continued*

$P_1p_3 \times P_1p_3$

Theoretical ratio		35	1
Pedigree Number	Parent	P	p
20209	19743(8) self	156	5
20210	19743(9) self	296	13
20217	19743(15) self	234	6
20230	19781(18) self	95	3
20234	19794(26) self	170	1
Totals		6595	225
Calculated		6630.6	189.4

Dev. $= \frac{35.6}{9.15} = 3.891$
P.E.

Totals of tables VIa and VIIb		9199	225
Calculated		9162.8	261.8

Dev. $= \frac{36.8}{10.76} = 3.420$
P.E.

a: PARENTS TRIPLEX (P_3p) FOR PURPLE FACTOR

$P_1p \times P_1p$

Theoretical ratio		∞	0
Pedigree Number	Parent	P	p
1998	17108(9) self	151	0
19743	1998(16) self	479	0
19389	19108(4) self	650	0
Totals		1280	0
Calculated		1280	0

$P_3p \times p_4$ and reciprocal

Theoretical ratio		∞	0
Pedigree Number	Parent	P	p
1999	17108(9)×17431(1)	66	0
19390	19108(4)×1989(13)	53	1
19391	1989(13)×19108(4)	41	0
Totals		160	1
Calculated		161	0

c: PARENTS SIMPLEX (Pp_3) FOR PURPLE FACTOR; PARENTS MARKED WITH DAGGER (†) WERE FROM PEDIGREES WHICH HAD SHOWN A 35:1 RATIO FOR PURPLE AND WHITE

$Pp_3 \times Pp_3$

Theoretical ratio		3	1
Pedigree Number	Parent	P	p
17103	16848(2)† self	77	19
1996	16848(2)† self	78	26
17106	16848(3)† self	15	3
17133	16848(15)† self	21	3
17459	17103(10)×17103(9)	50	10
17414	17103 (15) self	3	1
17460	17103(15)×17103(9)	44	16
17415	17103(16) self	42	22
17416	17103(18) self	120	35
17417	17103(20) self	100	26
17422	17106(7) self	48	14

$Pp_3 \times p_4$ and reciprocal

Theoretical ratio		1	1
Pedigree Number	Parent	P	p
1997	16848(2)×17438(3)	22	31
17484	16848(3)×17135(13)	10	11
17451	17103(9)×17103(5)	9	5
17444	17103(5)×17103(9)	27	16
17461	17140(3)×17103(9)	23	27
17494	17103(10)×17140(37)	9	15
17452	17103(12)×17103(5)	10	6
17495	17103(12)×17140(37)	8	8
17490	17103(13)×17140(37)	27	21
17453	17103(14)×17103(5)	13	13
17454	17103(15)×17103(5)	12	19
17490	17103(15)×17135(13)	5	4
17498	17103(15)×17140(37)	22	30
17499	17103(16)×17140(37)	68	75
17491	17103(16)×17135(13)	13	12

TABLE VII—*Continued*

$Pp_3 \times Pp_3$

Theoretical ratio		3	1
Pedigree Number	Parent	P	p
17424	17106(11) self	39	10
17426	17106(15) self	37	9
17435	17133(20) self	73	26
19106	17123(4)† self	61	19
19350	1996(3) self	317	117
19351	1996(4) self	372	117
19363	1996(7) self	375	128
19367	1996(11) self	67	22
19370	19106(2) self	252	86
19371	19106(4) self	81	39
19376	19102(6)† self	37	10
19384	19108(1)† self	464	147
19388	19108(2)† self	359	145
19396	19108(8)† self	240	87
19399	19108(9)† self	509	167
19401	19108(10)† self	450	165
19405	19108(11)† self	405	151
19412	19108(14)† self	80	32
19417	19110(3) self	255	91
19757	19354(4)† self	52	19
19762	19359(5)† self	69	25
19764	19373(2)† self	91	35
19777	19392(3)† self	75	22
19787	19413(3)† self	122	29
19791	19410(2) self	26	8
19794	19350(1) self	132	52
19795	19350(2) self	135	57
19797	19350(4) self	50	17
19798	19350(5) self	85	29
19799	19351(1) self	59	18
19800	19351(2) self	129	33
19803	19351(5) self	81	31
19804	19363(1) self	62	24
19805	19363(2) self	51	20
19806	19363(3) self	115	40
19810	19367(2) self	56	26
19811	19367(3) self	76	21
19847	19396(5) self	111	43
19854	19401(2) self	94	33
19855	19401(3) self	53	18
19856	19401(4) self	187	57
19858	19405(1) self	92	36
19863	19412(1) self	96	31
19864	19412(2) self	37	9
19866	19412(4) self	56	16
19868	19417(1) self	108	57
19870	19417(3) self	124	49
20235	19794(26) self	46	15
Totals		7547	2619
Calculated		7624.5	2541.5

$$\text{Dev.} = \frac{102.5}{\text{P.E.}_{93.13}} = 1.101$$

$Pp_4 \times p_4$ and reciprocal

Theoretical ratio		1	1
Pedigree Number	Parent	P	p
17502	17106(11)×17140(37)	4	2
19107	17123(4)×17438(3)	26	36
19352	1996(4)×1986(2)	23	25
19353	1989(17)×1996(4)	20	13
19364	1996(7)×1986(1)	20	15
19365	1986(1)×1996(7)	8	6
19366	1996(7)×1989(11)	23	23
19369	1989(10)×1996(11)	2	4
19385	1986(13)×19108(1)	41	25
19386	19108(1)×1989(1)	52	41
19387	1989(1)×19108(1)	21	15
19397	19108(8)×1989(6)	20	26
19398	1989(6)×19108(8)	11	7
19400	1986(10)×19108(9)	51	48
20238	1989(6)×19108(9)	15	12
19402	1986(7)×19108(10)	8	4
19403	19108(10)×1989(11)	4	5
19404	1989(11)×19108(10)	12	11
19406	19108(11)×1986(4)	18	20
19407	19108(11)×1989(17)	3	7
19408	1989(17)×19108(11)	36	44
Totals		696	682
Calculated		689	689

$$\text{Dev.} = \frac{7}{\text{P.E.}_{12.52}} = 0.559$$

d: PARENTS NULLIPLEX (p_4) FOR PURPLE FACTOR

$p_4 \times p_4$

Theoretical ratio		0	∞
Pedigree Number	Parent	P	p
17412	17103(5) self	0	114
17419	17106(3) self	0	4
17438	17140(3) self	0	21
1985	17394(6) self	0	101
1986	17412(2) self	0	41
1987	17427(9) self	0	167
1988	17431(1) self	0	70
1989	17438(3) self	0	103
19740	1985(4) self	0	74
19741	1986(1) self	0	72
19742	1989(6) self	0	81
Totals		0	848
Calculated		0	848

ples for an exact 35:1 ratio. If the purples of table VI*a* are included, we have 9,199 purples to 225 whites, which gives too many purples. The true value for the breeding data in hand lies somewhere between the two ratios, and approaches closely to the 35:1 ratio found from selfing duplex parents in race B. The back-crosses give totals reasonably close to a 5:1 ratio, although without the addition of the data from table VI*a* they are slightly high in whites.

The simplex (Pp_3) parents listed in table VII*c* gave by selfing 7,547 purples and 2,619 whites, which is a ratio with a deviation from 3:1 only about one time the probable error, while the back-cross with 696 purples to 682 whites has a deviation from a 1:1 ratio of less than the probable error. Table VII*d* shows that the whites breed true.

In addition, certain relations are shown by the data in race A that are lacking in the present data in race B. Thus we are able to identify the three triplex (P_3p) parents listed in table VII*a*. Triplex parents should breed apparently true the first generation whether selfed or back-crossed, but some of their offspring should be duplex (P_2p_2) as shown by table IV, and consequently should throw the recessive whites in a later generation. The individual plant 17108 (9) when selfed gave 151 offspring, all purple, and when back-crossed gave 66 offspring, also all purple, the two pedigrees together amply establishing the fact that the parent must have been quadriplex (P_4) or triplex (P_3p). One of its immediate offspring from selfing no. 1908 (16) when selfed gave a progeny of 479 all purple individuals, and likewise must have been either quadriplex or triplex. That both these individual plants in question from table VII*a* were in reality triplex is shown by the fact that in the next generation four (parents 19743 (1), (8), (9), and (15), table VII*b*) out of 19 individuals segregated in a 35:1 ratio when selfed. (The non-segregating 19743 individuals are shown in table VI.) That plant 19108 (4) was triplex is shown by the fact that one of its offspring (19389 (3), table VII*b*) was duplex and segregated in a 35:1 ratio. The fact that this triplex parent produced one white individual out of 95 offspring in a back-cross where only purples were expected, may not have been due to an experimental error, but may have been brought about by the same

causes which are responsible for the appearance of exceptional *inermis* individuals, to be discussed later.

A sufficient number of individual offspring from the different types of matings has not yet been tested to determine whether the distribution of genetic types among the purples follows the expectation shown in table IV. As indicated by the asterisks in table VII*b*, and by the daggers in VII*c*, duplex (P_2p_2) individuals occur in the offspring of simplex (Pp_3) parents, and simplex individuals in the offspring of duplex parents.

Under greenhouse conditions in the winter it is often possible to distinguish homozygous from heterozygous purples among diploids by the obviously greater amount of pigment in the flowers of the former. Such a distinction, however, cannot be made readily under conditions in the field in summer. In tetraploid cultures which are segregating for color there has always been a considerable amount of variation in the strength of pigment, in the flowers of different purple plants. A preliminary attempt was made to grade, by means of the color top, flowers of the parents represented in table V. The matching of the color was difficult, and only a limited number of flowers was available for use from some of the plants when the tests were made. Despite the crudeness of the tests, however, a comparison of the color grades with the genetic constitution of the plants as determined a year later showed a rough correspondence between the amount of pigment in the flowers and the number of chromosomes in the plant's nucleus carrying the gene for purple. The distinction was best between the genetic classes Pp_3 and P_2p_2, and, although plants whose genetic constitution was either P_3p or P_4 averaged a trifle darker than the P_2p_2 plants, there was considerable overlapping of the color grades of the two types. It is possible that with more accurate methods of estimating the amount of purple in the flowers it would be feasible to determine the genetic constitution of tetraploid plants without the necessity of resorting to breeding tests. Tetraploids of lines 10 and 18 apparently correspond in flower color to the homozygous purple diploids from which they arose. In other words, the P_4 condition in a tetraploid seems to have the same external effect as the P_2 condition in a diploid.

If we sum the evidence in regard to races B and A, it is apparent that, so far as the inheritance of purple and white flower color is concerned, the data from the tetraploid race A, with over 22,000 individuals recorded, amply confirm the conclusions reached from the more carefully planned analysis of race B, and are in accord with the theoretical ratios derived from an assumption of random assortment of the four chromosomes concerned.

Inheritance of spines on capsules.—While, as has been shown, the inheritance of purple pigmentation is according to expectation, the inheritance of spines on the capsules, tabulated in table VIII, shows significant deviations from the theoretical ratios. In all cases where both armed and *inermis* offspring should occur from a given mating, the number of *inermis* individuals is greater than the calculated values would lead one to expect. The excess of the *inermis* recessives apparently is not significant in the 1:1 and the 11:1 ratios when these are considered by themselves. The other ratios, however, show a statistically significant excess of the recessives with a deviation of over seven times the probable error for the 3:1 ratio.

The most striking deviation is the occurrence of recessives where none at all would be expected. Thus, in table VIII*a* the parents B(2), B(4), and B(5), which are doubtless triplex (A_3a) for the armed factor, are seen to produce *inermis* offspring both when crossed with recessives (a_4) and when crossed with simplex (Aa_3) individuals. The gametes expected from a triplex parent are A_2 and Aa in equal numbers. The latter type, containing one chromosome with the dominant (A) and one chromosome with the recessive factor (a), alone concerns us here. The problem apparently is to account for the elimination of the chromosome A, which if present causes the zygote which it helps form to show the dominant character. This elimination of A could most readily be explained by non-disjunction, or detachment, which would ultimately bring about a zygote with a deficiency in the armed-*inermis* set of chromosomes. Such an explanation cannot well apply to the two exceptional recessives studied, which had each 48 somatic chromosomes as determined from division figures in the pollen mother cells. Another possible explanation would involve an irregularity in

TABLE VIII

TETRASOMIC INHERITANCE OF ALLELOMORPHIC CHARACTERS ARMED (*A*) AND *inermis* (*a*) CAPSULES IN TETRAPLOID RACES B AND C (SEE LEGEND OF TABLE V)

a: PARENTS QUADRIPLEX (A_4) OR TRIPLEX (A_3a) FOR ARMED FACTOR

A_4 or $A_3a \times A_4$ or A_3a			A_4 or $A_3a \times a_4$ and reciprocal			A_4 or $A_3a \times Aa_3$ and reciprocal			A_4 or $A_3a \times Aa_3$ and reciprocal		
Theoretical ratio	∞	o	Theoretical ratio	∞	o	Theoretical ratio	∞	o	Theoretical ratio	∞	o
Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*
B(2) self	303	o	B(2)×B(10)	71	1	B(7)×B(2)	165	3	B(9)×B(2)	166	o
			B(2)×B(11)	19	o	B(14)×B(2)	84	1	B(13)×B(2)	35	o
			B(10)×B(2)	8	o	B(17)×B(2)	97	2	B(21)×B(2)	118	o
			B(11)×B(2)	19	o	B(18)×B(2)	135	1	B(22)×B(2)	67	o
						B(25)×B(2)	93	o	B(29)×B(2)	53	o
						B(26)×B(2)	77	o			
						B(27)×B(2)	29	o			
B(4) self	283	o	B(4)×B(10)	69	1						
B(4)×B(2)	103	o									
B(5) self	326	o	B(5)×B(11)	71	4						
B(5)×B(2)	90	o									
B(6) self	282	o				B(6)×B(7)	39	o			
B(8) self	277	o				B(7)×B(8)	28	o			
B(19) self	180	o									
B(19)×B(2),	53	o									
Totals	1897	o	Totals	257	6	Totals	747	7	Totals	439	o
Calculated	1897	o	Calculated	263	o	Calculated	754	o	Calculated	439	o

b: PARENTS DUPLEX (A_2a_2) FOR ARMED FACTOR

$A_2a_2 \times A_2a_2$			$A_2a_2 \times a_2a_2$ and reciprocal			$A_2a_2 \times Aa_2$ and reciprocal			$A_2a_2 \times A_2a$ or A_2 and reciprocal		
Theoretical ratio	35	1	Theoretical ratio	5	1	Theoretical ratio	11	1	Theoretical ratio	∞	0
Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*
B self	230	6									
C self	390	15									
B(3) self	249	11	B(3)×B(10)	71	23	B(14)×B(3)	70	7			
			B(3)×B(11)	21	1	B(20)×B(3)	45	3			
			B(10)×B(3)	16	7	B(27)×B(3)	37	0			
			B(11)×B(3)	27	10	B(7)×B(3)	109	15			
B(9) self	552	21	B(9)×B(10)	96	36				B(9)×B(2)	166	0
B(13) self	571	21	B(13)×B(10)	118	29	B(13)×B(7)	15	0	B(13)×B(2)	35	0
B(13)×B(3)	22	1				B(7)×B(13)	43	7			
B(13)×B(9)	55	2									
B(21) self	327	10	B(21)×B(10)	44	10				B(21)×B(2)	118	0
B(22) self	251	13	B(22)×B(10)	29	6				B(22)×B(2)	67	0
B(29) self	254	7	B(29)×B(10)	95	15				B(29)×B(2)	53	0
B(30) self	240	6	B(30)×B(11)	1	0						
B(30)×B(3)	142	3									
B(31) self	94	2									
Totals	3383	118	Totals	518	137	Totals	319	32	Totals	439	0
Calculated	3403.7	97.3	Calculated	545.8	109.2	Calculated	321.7	29.3	Calculated	439	0
$\frac{\text{Dev.}}{\text{P.E.}} = \frac{20.7}{6.56} = 3.155$			$\frac{\text{Dev.}}{\text{P.E.}} = \frac{27.8}{6.43} = 4.323$			$\frac{\text{Dev.}}{\text{P.E.}} = \frac{2.7}{3.49} = .774$					

TABLD VIII—*Continued*

c: PARENTS SIMPLEX (Aa_3) FOR ARMED FACTOR

$Aa_3 \times Aa_3$			$Aa_3 \times a_4$ and reciprocal			$Aa_3 \times A_4a_3$ and reciprocal			$Aa_3 \times A_3a$ or A_4 and reciprocal		
Theoretical ratio	3	1	Theoretical ratio	1	1	Theoretical ratio	11	1	Theoretical ratio	∞	0
Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*
B(7) self	192	111	B(7)×B(10)	36	29	B(7)×B(3)	109	15	B(7)×B(2)	165	3
			B(7)×B(11)	15	12	B(7)×B(13)	43	7	B(7)×B(8)	28	0
						B(13)×B(7)	15	0	B(6)×B(7)	39	0
B(14) self	89	38				B(14)×B(3)	70	7	B(14)×B(2)	84	1
B(17) self	232	105							B(17)×B(2)	97	2
B(17)×B(7)	10	3									
B(18) self	256	110	B(18)×B(10)	27	22				B(18)×B(2)	135	1
B(20) self	169	56				B(20)×B(3)	45	3			
B(25) self	239	83	B(25)×B(10)	28	32				B(25)×B(2)	93	0
B(26) self	82	36	B(26)×B(10)	22	22				B(26)×B(2)	77	0
B(27) self	68	28	B(27)×B(11)	16	27	B(27)×B(3)	37	0	B(27)×B(2)	29	0
Totals	1337	570	Totals	144	144	Totals	319	32	Totals	747	7
Calculated	1430.2	476.8	Calculated	144	144	Calculated	321.7	29.3	Calculat d	754	0
Dev./P.E. $= \frac{93.2}{12.76} = 7.304$			Dev./P.E. $= \frac{0.0}{5.72} = 0.00$			Dev./P.E. $= \frac{2.7}{3.49} = 0.774$					

d: PARENTS NULLIPLEX (a_4) FOR ARMED FACTOR

$a_4 \times a_4$			$a_4 \times Aa_4$ and reciprocal			$a_4 \times A_4a_4$ and reciprocal			$a_4 \times A_3a$ or A_4 and reciprocal		
Theoretical ratio	0	∞	Theoretical ratio	1	1	Theoretical ratio	5	1	Theoretical ratio	∞	0
Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*	Pollination	*A*	*a*
B(10) self.....	0	91	B(7)×B(10).....	36	29	B(10)×B(3).....	16	7	B(10)×B(2).....	8	0
			B(18)×B(10).....	27	22	B(3)×B(10).....	71	23	B(2)×B(10).....	71	1
			B(25)×B(10).....	28	32	B(9)×B(10).....	96	36	B(4)×B(10).....	69	1
			B(26)×B(10).....	22	22	B(13)×B(10).....	118	29			
						B(21)×B(10).....	44	10			
						B(22)×B(10).....	29	6			
						B(29)×B(10).....	95	15			
B(11) self.....	0	233	B(7)×B(11).....	15	12	B(11)×B(3).....	27	10	B(11)×B(2).....	19	0
			B(27)×B(11).....	16	27	B(3)×B(11).....	21	1	B(2)×B(11).....	19	0
						B(30)×B(11).....	1	0	B(5)×B(11).....	71	4
Totals.....	0	324	Totals.....	144	144	Totals.....	518	137	Totals.....	257	6
Calculated	0	324	Calculated.....	144	144	Calculated.....	545.8	109.2	Calculated.....	263	0
			Dev./P.E. $= \frac{0.0}{5.72} = 0.00$			Dev./P.E. $= \frac{27.8}{6.43} = 4.323$					

disjunction in the second division of the gamete mother cell. After the reduction of a A_3a somatic cell, one of the resulting disomes would have the formula Aa. At the next division normally the two halves of the chromosomes Aa go to the opposite poles, as indicated by the separating line in the fraction $\frac{Aa}{Aa}$. Since the four chromosomes are homologous, it is possible that the separation sometimes would be between chromosomes with unlike factors, as if the separating line in the fraction were made vertical $\left(\begin{matrix}A|a\\A|a\end{matrix}\right)$, producing gametes of the formula AA and aa. The latter type, when mated to a gamete of similar constitution, would give the exceptional recessive. This would have the same effect as a double non-disjunction at this stage in the armed-*inermis* chromosomal set, and would also be brought about if crossing over should take place in the second division of the gamete mother cells without regard to the division mates.

It will be noted that exceptional recessives form slightly over 2 per cent of the offspring when the triplex parents B(2), B(4), and B(5) are back-crossed to a_4, while when the parent B(2) is crossed to Aa_3, only about 1 per cent of exceptions are produced. This difference in percentage of occurrence is to be expected from the fact that Aa_3 parents have only half of their gametes of the formula a_2. Inasmuch as A_2a_2 parents have only a sixth of their offspring with the formula a_4, we should expect a cross between a triplex parent (A_3a) and a duplex parent (A_2a_2) to give only one-third of 1 per cent exceptions. It is not surprising, therefore, that we did not find them in the pedigrees which totaled only 439 individuals from this type of mating. The likelihood of ever getting exceptions from selfing a triplex plant is extremely slight, since it would involve the union of an exceptional egg cell with an exceptional male gamete. A larger number of offspring from selfing triplex parents, however, should yield a small percentage of exceptions, since they are carried by the pollen as well as by the egg cell, as indicated by the data on B(2) in table VIII*a*.

Similar *inermis* exceptions are produced by the mutant Cocklebur, whereas no white exceptions have been produced by

the mutant Poinsettia. Cocklebur and Poinsettia, it will be remembered (**5**), are $(2n+1)$ mutants with an extra chromosome belonging respectively to the armed-*inermis* and to the purple-white chromosomal set. It is evident, therefore, that the exceptions are in some way conditioned by the peculiarity of the armed-*inermis* chromosomal set in comparison with the purple-white set.

Dihybrid ratio of color and spines.—The data in regard to the inheritance of color and spines in race B have been summarized separately in tables V and VIII, although each mating involved the possible segregation of both pairs of characters. Since no linkage relations were observed, it will not be desirable to analyze all

TABLE IX

TETRASOMIC INHERITANCE OF CHARACTER PAIRS PURPLE (*P*) AND WHITE (*p*) FLOWER COLOR, AND ARMED (*A*) AND *inermis* (*a*) CAPSULES; DIHYBRID RATIO: PARENT DUPLEX FOR BOTH COLOR AND SPINES ($P_2p_2A_2a_2$)

Theoretical ratio	1225	35	35	1
Pollination	*PA*	*Pa*	*pA*	*pa*
B self	226	6	10	0
C self	378	15	12	0
B(9) self	534	21	18	0
B(21) self	312	9	15	1
B(29) self	246	7	8	0
Total	1696	58	63	1
Calculated	1718.4	49.1	49.1	1.4

the pedigrees in respect to their dihybrid segregation. The segregation of parents duplex in respect to both color and spines ($P_2p_2A_2a_2$) is shown in table IX. The dihybrid ratio for selfing doubly duplex tetraploids is 1,225:35: :35:1. The ratio gives an expectation of one double recessive in 1,296 individuals. The figures in the table show a reasonably close approximation to the calculated values. If the character pair many and few nodes had been more readily classified, we would have had the opportunity of comparing the offspring from triply duplex parents with the calculated trihybrid ratio for tetraploids, which is 42,875:1,225:1,225:1,225:35:35:35:1. A triple recessive might be expected to appear, therefore, once in 46,656 individuals.

Discussion

This paper deals primarily with the inheritance of two Mendelian characters in tetraploid Daturas, and with the behavior of the chromosomes only so far as seems necessary in order to obtain a basis for their rather novel behavior. A detailed consideration of the more purely cytological problems involved in the association of homologous chromosomes into tetrasomes instead of into the more common disomes, is deferred to a later paper. The breeding data on *Datura* not only indicate random assortment of chromosomes as the primary cause of the ratios obtained, but also provide in these ratios a test for tetraploidy which may be applied in forms in which Mendelian characters are available.

A study of tetraploidy unavoidably brings up a consideration of the rôle which duplication of homologous chromosomes may have played in the evolution of plants and animals. The question has already been discussed in connection with chromosomal mutants in *Datura* (**4**), and need not be considered in detail in the present paper. Many species in nature have been suspected of being tetraploids of closely related forms, but so far as we are aware, there is available in such cases neither breeding data nor cytological evidence beyond the mere chromosomal counts. If tetraploidy has been a method of evolution, either we should expect to find tetraploid species maintaining themselves at the present time in nature, or we should expect tetraploidy to be a transitional stage giving way ultimately to a "double diploid" condition. If the latter conception is the correct one, we might expect in the course of time to have the four homologous chromosomes in the various sets of the tetraploid *Datura* form two sets of two each. The total chromosomal counts would not be altered by this change, but some of the breeding behavior would be different. Thus a plant which possessed two chromosomes with the gene for purple would either breed true for purple, if these chromosomes were in the same set, or would give a 15:1 ratio from selfing, if these chromosomes were in different sets.[5] In the latter case we should probably

[5] The single parent B(24), previously mentioned, was at first thought possibly to represent a double diploid, since its offspring showed an approach to a 15:1 ratio in regard to both color and spines. Tests of individuals in the next generation, however, have shown that, whatever the condition in the plant B(24) may have been, we have failed to establish a double diploid race.

speak of "duplicate genes," a designation which has been applied to the factors in a 15:1 ratio, without a knowledge of the chromosomal evolution of the form under investigation. While on the one hand certain of the heterozygous purple individuals in a tetraploid culture showing segregation in a 3:1 ratio (in which the genetic types of purple are $P_2p_2+2\ Pp_3$) should in a later generation when selfed throw a 35:1 ratio, on the other hand purples in a 3:1 ratio of a double diploid (in which the genetic types are $PPp'p'+2Ppp'p'$), if heterozygous, can segregate when selfed only in a 3:1 ratio.

The cytological distinction between tetraploids and double diploids would lie in the fact that the latter would have two instead of four chromosomes in a set, although they would have twice as many sets as the former. If the individual chromosomes in the haploid condition differ in size one from the other, then in double diploids it should be possible to arrange the sizes of the chromosomal sets in groups of two, each two sets in a group corresponding to the original set from which they had been derived. With this in mind, an investigation was made of the chromosomes of shepherd's purse, in which SHULL (**23**) has demonstrated the presence of duplicate genes. Unfortunately, however, it was not found possible to establish size classes among the chromosomes of this plant. It may be said that no decisive evidence has been obtained as yet in *Datura* or in other forms for a change from tetraploidy to double diploidy, although a number of facts already suggested indicate that such a change is not an improbable step in evolution.

In a tetraploid, as we have defined the term, there are twice as many chromosomes as in the corresponding diploid, and each chromosome of the latter is represented twice. Apparently this doubling of the chromosomal material brought about by doubling the number of chromosomes is responsible for the general increase in size of cells and various organs characteristic of tetraploids. It is conceivable, however, that the same increase in chromosomal material might be brought about by a more or less permanent union of homologous chromosomes. The plant resulting from such a process would show the diploid number, but the compound chromosomes thus formed might be expected to be larger than those in the parent form. At the present stage of our knowledge, a

discussion of this subject is perhaps somewhat speculative. It must be said, however, that certain reported cases of gigantism in which a doubling of chromosomal number has not occurred suggest the possiblity of chromosomal unions. Thus FARMER and DIGBY (12) show that, although the sterile giant *Primula kewensis* has the diploid number, the chromosomes are twice the size of those in the fertile *P. kewensis*, which has the tetraploid number. They conclude that the doubled number of chromosomes of the latter may be attributed to a transverse fission of the normal chromosomes. Conceivably the sterile *P. kewensis* might be a "compound diploid" with compound and therefore larger chromosomes formed by the union of chromosomes from its two parents, *P. floribunda* and *P. verticillata*. The size of chromosomes in these two parent species, therefore, is of the greatest interest, but unfortunately FARMER and DIGBY give measurements for the chromosomes only of their hybrid offspring *P. kewensis*. Miss DIGBY (11), however, gives camera drawings under the same magnification, showing the polar view of chromosomes at "the equatorial plate of the first meiotic division" for the two parents *P. floribunda* (fig. 34) and *P. verticillata* (fig. 52), as well as for the sterile hybrid *P. kewensis* (fig. 65) and the fertile hybrid *P. kewensis* (fig. 96). These comparable figures show the nine chromosomes of each of the two parents and the eighteen chromosomes of the fertile hybrid to be essentially the same size, but the nine chromosomes of the sterile hybrid to be distinctly larger. If the sizes in the figures are typical of the respective forms, they offer an argument for union of chromosomes in the sterile hybrid *P. kewensis*.

In addition to his tetraploid *P. sinensis*, GREGORY (15) has reported a giant form in this species with the diploid number but with chromosomes of larger size. It will not be desirable to cite further instances of *gigas*-like forms which fail to show the tetraploid number of chromosomes. Enough has been said to show that union of homologous chromosomes is a conceivable process, which might be expected to duplicate the effects upon the plant of a doubling of the individual chromosomes and their retention throughout the plant as discrete units.

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Summary

1. Tetraploid ($4n$) plants in *Datura Stramonium*, in comparison with diploid ($2n$) plants, are characterized, among other morphological differences, by spherical instead of ovate capsules and by larger pollen grains.

2. Pollen of tetraploids is relatively good, containing only slightly more shriveled grains than that of diploids.

3. The chromosomes in the pollen mother cells are typically arranged in twelve sets of four each, and at the reduction division in about 70 per cent of the cases investigated assort 24–24, in about 30 per cent of the cases assort 23–25, and rarely 22–26.

4. Tetraploids have occurred spontaneously at least six times, three of them in three distinct lines and three of them as plants derived from crosses between two other lines.

5. Since one of the tetraploids arose in an F_2 from the cross $P_2A_2 \times p_2a_2$, and itself was shown to be $P_2p_2A_4$, the origin of tetraploidy in this case cannot be attributed to a doubling of the chromosomes before reduction. Since two other tetraploids arose in the F_1 from the cross $P_2a_2 \times p_2A_2$ and were themselves $P_2p_2A_2a_2$, in these two cases the origin of tetraploidy cannot be attributed to apogamy of a $4n$ egg cell. Other methods of origin are suggested.

6. In comparison with diploids, tetraploids (whether selfed or crossed with other tetraploids) set a relatively small proportion of capsules, which contain a relatively small number of seeds.

7. Crosses between tetraploids and diploids apparently are impossible when the diploid is the female; when the tetraploid is the female, an average of one viable seed has been obtained from about four pollinations. The offspring from this cross have been diploids, triploids, and $(2n+1)$ mutants.

8. Genetic and phenotypic ratios for quadriplex (A_4), triplex (A_3a), duplex (A_2a_2), simplex (Aa_3), and nulliplex (a_4) parents,

selfed and crossed *inter se*, are derived from the assumption of a random assortment of chromosomes at the reduction division.

9. Tabulations of breeding data in some 37,400 individuals for tetrasomic inheritance of the Mendelian pair purple-white flower color confirm the theoretical ratios, and justify the assumption of random assortment of the chromosomes which carry the genes for purple pigmentation.

10. The inheritance of the pair armed-*inermis* is in accord with the inheritance of the purple-white pair, aside from the production of about 2 per cent exceptional recessive offspring from A_3a parents when back-crossed to a_4 individuals. A possible explanation is suggested but no experimental evidence on the question is as yet available.

11. The possible relation of tetraploidy to the evolution of plants is discussed, and breeding and cytological tests are given to distinguish tetraploidy from "double diploidy." Union of homologous chromosomes is suggested as a possible cause of *gigas*-like forms without the double number of chromosomes.

STATION FOR EXPERIMENTAL EVOLUTION
COLD SPRING HARBOR, N.Y.

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LEAVES OF THE GRAMINEAE[1]

AGNES ARBER

(WITH PLATES XXVIII–XXX)

The anatomical structure of the leaves of the Gramineae has been the subject of considerable work during the last sixty years. The attention of botanists has been drawn to it, partly on account of its intrinsic interest, and partly because of the economic importance of the family, which has made it desirable to be able to identify its members in cases when leaves alone are available for examination. The present paper does not undertake a detailed descriptive anatomical study of grass leaves, since the work of DUVAL-JOUVE (**16, 17, 18**), PÉE-LABY (**23**), LEWTON-BRAIN (**22**), BRANDIS (**9**), and SABNIS (**25**) has rendered such an attempt superfluous; its object is rather interpretative. In a paper published in 1918 (**1**), the view is supported that the leaves of monocotyledons are equivalent to the leaf base and petiole (or leaf base alone) of the dicotyledon, the lamina being unrepresented. I included the Gramineae in this generalization, but did not consider them apart from the rest of the class. More recently BUGNON (**13**), in a memoir upon the leaves of grasses, has disputed this conception of the phyllodic nature of these organs, and has also called in question the interpretation of the grass seedling put forward some years ago by Miss SARGANT and myself (**27**). In the present paper it is proposed to reconsider the morphology of the foliage, seedling, and inflorescence leaves of the Gramineae, more particularly in connection with BUGNON's criticisms.

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[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher-Warr Studentship of the University of London.

Mature foliage leaf

The leaf of the grass consists essentially of a sheath and limb, with a ligule marking the line of junction of the two. The sheath may be open to the base, or it may be closed and tubular for a part or the whole of its length (**15**). Figs. 19 and 20*A* show that the leaf base may more than inclose the circumference of the stem, forming a "wrap-over." On the other hand, fig. 18*A* gives an example of a tubular sheath. Fig. 1 represents a typical grass leaf, that of a species of *Lolium*, in which the sheath (*sh*) is very long, and is succeeded by a long linear limb (*l*). This limb, in certain grasses, may attain a considerable size. In cultivated examples of *Panicum palmifolium* it is said to reach a length of 1 m., and to be as wide as one's hand (**21**). The limb of the grass leaf may be modified, as regards its form, in three distinct ways. Sometimes the greater part of it remains more or less uniform in width, while its shape is complicated by the production of basal auricles (*Hordeum Zeocriton*, fig. 3*A*). In other cases (such as *Panicum plicatum*, fig. 25*C*) the limb is broader, and shows a fanlike folding. In a third set of cases (certain bamboos, fig. 2) the limb is differentiated into a proximal stalk (*pet*) and a distal bladelike region (*ps. l*). This differentiation may be compared, as DUVAL-JOUVE has suggested (**17**), with that in the awn of *Avena sterilis*, in which the proximal column and distal "subule" (fig. 4*c*, *s*) are very distinct.

My view of the morphology of the grass leaf is that, despite the minor modifications to which it is liable, the basal sheath in all cases is equivalent to the same region in the dicotyledon, while the limb corresponds to the petiole of the dicotyledon, the lamina being absent. In such a case as the *Bambusa* shown in fig. 2, the "blade" is considered as a pseudo-lamina, resulting from a transformation of the distal region of the petiole.[2] It is true that the thin, flat, flexible limb, which characterizes most grass leaves, does not at first glance suggest a petiole, but this difficulty is more apparent than real, for in the leaves of *Cymodocea*, *Sagittaria*, and

[2] BUGNON (**13**) does not convey quite an accurate impression when he cites me as regarding the limb of the grass leaf as "une néoformation." I do not interpret it as a new formation, but as a modification of a pre-existing organ, the petiole. I fear that this point was not expressed as clearly as it should have been in my 1918 paper (**1**).

Triglochin (3) we see how readily the change from a "radial," petiole-like limb to a thin, ribbon-like limb may come about.

BUGNON's view of the nature of the grass leaf is sharply opposed to mine. He treats the limb of the grass as equivalent to the basal sheath of a dicotyledon, and the sheath of the grass leaf as a new formation ("un organe nouveau, . . . soulevant au-dessus de lui l'organe primitif devenu fonctionnellement un limbe"). It is difficult to present individual arguments against this view, since it removes the grass leaf from the ordinary sphere of comparison. It can only be said that, on general grounds, we should be reluctant to admit any morphological theory which postulates the production of some entirely new organ. The only evolutionary changes among flowering plants about which anything is known are those concerned with the suppression, fusion, or modification of existing structures. I should not venture, of course, to say that no new organ is ever formed, but, in any question of morphological interpretation, this assumption should only be used as a last resort, when all other possible explanations have been weighed and found wanting. Another general argument against BUGNON's theory, if I understand it aright, is that it has the effect of isolating the leaf structure of grasses from that of the other monocotyledons. It seems scarcely possible, for instance, to apply it to the many "radial" leaves met with in this class. The writer's study of grass leaves has led to the conclusion that it is fundamentally unsound to treat them as if they were organized on a different plan from the other monocotyledons. They show remarkable parallelisms with the leaves of other cohorts, and I do not think any theory can safely be used to interpret them, unless it brings them into line with the other monocotyledons. On the view here advocated, namely, that they are phyllodes equivalent to the leaf base and petiole of the dicotyledon, the leaves of the Gramineae are not relegated to a separate category, but fall into place among those of the other monocotyledons. The parallelism in form between grass leaves and those of various other monocotyledonous families is very close. A sheath, ligule, and straplike linear limb, similar to the corresponding organs in a typical grass leaf, may be found, for instance, in *Cymodocea nodosa* of the Potamogetonaceae (3). Even the more peculiar

of the grass leaves find their counterpart in other families. For example, the fanlike limb of *Panicum plicatum* (fig. 25*C*) recalls that of *Joinvillea elegans* of the Flagellariaceae (**8**, fig. 17). The venation of the auricles in *Hordeum* (fig. 3*B*) also, in which marginal bundles from the limb execute a *V*-like bend, resembles that of the auricled limb base of *Monochoria* among the Pontederiaceae (**5**). The chief difference between the two cases is that in *Monochoria* it is only the distal part of the petiole that forms the expanded limb, whereas in *Hordeum*, as in the majority of grasses, the whole of the ancestral petiole is represented by a thin narrow limb, so that the auricles are placed immediately above the sheath. The parallelism between grass leaves and those of other monocotyledons extends even to small details. Celakovský (**14**) has pointed out that the leaves of *Ammophila* terminate in a cylindrical hoodlike apex, the smoothness of whose surface is due to the dying out of the grooves which furrow the main part of the limb. A similar apex is met with in the leaves of certain palms, and, as shown elsewhere (**7**), this peculiarity is due to the same cause as in *Ammophila*.

Young foliage leaf

The limb of the mature foliage leaf of many grasses is characterized by longitudinal ridges and grooves, usually involving the upper surface alone. Such a form of leaf, which is sketched in transverse section for species of *Aira*, *Festuca*, and *Lygeum* in figs. 17*B*, 16*B*, and 23*B* and *C*, lends itself with particular ease to the folding or rolling which is such a familiar feature of many grass leaves. In order to follow the origin of this type of structure, serial sections through the plumular buds of seedlings, and through the leaf buds of mature plants have been examined. In the majority of the grasses studied the development follows a uniform course, as, for example, *Hordeum vulgare*. Fig. 11*A* shows a section through the plumular bud of the seedling, including also the coleoptile (*col*) and the top of the scutellum (*sc*). The second plumular leaf (l_2) is cut at its extreme base, at a level at which it forms a closed sheath, and is partially fused both with the leaf (l) outside it, and the leaf (l_3) inside it. Fig. 11*B–D* shows the further upward development of the leaf (l_2), drawn on a larger scale, and in a

position at right angles to that which it occupies in fig. 11*A*. Fig. 11*B* shows the first sign of the ligule (*lig*), which makes its appearance as a ventral flap inside each margin of the sheath. In higher sections the free margins of the ligule are cut nearer and nearer the median bundle, since the junction of sheath and limb is not horizontal, but curves downward toward either margin. In fig. 11*C*, for instance, the marginal part of the transverse section belongs to the limb (*l*) and the median part to the sheath (*sh*), the ligular flaps (*lig*) marking the limit between the two regions. The ventral surface of the leaf sheath region is quite smooth, but immediately above the detachment of the ligule this surface begins to be invaginated, the grooves occurring between the bundles. The onset of invagination in the limb region (*l*) can be seen in fig. 11*C*, and more markedly in fig. 11*D*. A closely similar history can be followed in the young leaves of *Triticum vulgare* (fig. 10*B–D*), and also in *Arrhenatherum avenaceum* var. *bulbosum* (fig. 6*A–C*), *Sorghum vulgare* (fig. 7*A*, *B*), and *Lolium multiflorum*. In *Nardus stricta* (fig. 18*A–C*) the sheath at the base is completely closed (fig. 18*A*), and when the limb is reached, the invaginations are few but deep (fig. 18*C*). Fig. 18*B* shows the detachment of the ligule, which appears one-sided, probably owing to a slight obliquity in the section. *Saccharum officinarum* (fig. 24*A*, *B*) and *Arundinaria spathiflora* (fig. 9*A–C*) are essentially similar to the cases just described, but differ in the relatively greater thickness of the median region of the leaf, especially in the upper part of the sheath. In *Arundinaria spathiflora*, at the level shown in fig. 9*B*, there is considerable meristematic activity on the dorsal side of the sheath in the median region. The young leaf of *Ammophila arundinacea* (*Psamma arenaria* Roem. et Schult.) offers particularly favorable material in which to follow the development of a grooved leaf. Bugnon (**13**) has given figures from serial sections through a bud showing the origin of the ligule, but some further drawings are included here (fig. 21*A–C*, fig. 22) to show the close packing of the leaves in the bud, and the unusual depth of the invaginations. It seems that the curious form which finally results (fig. 21*C*, fig. 22) is due, not only to invaginations, but also to the upward growth of the ridges left between them. These ridges, in the part of the young limb above the region which enwraps the apical bud,

apparently continue to grow until further development is checked by their meeting one another.

In addition to the grass leaves which are ridged and furrowed on the ventral side only, there are certain genera in which there are grooves on both surfaces. Fig. 20*A–F* shows the structure of a young leaf in one of these cases, *Gracilea Royleana*. In fig. 20*A* the extreme base of the leaf is shown. It is still incompletely free from the axis, but even at this level its margins (*sh*) overlap; in other words, the leaf base forms more than one complete turn round the axis. In fig. 20*B* the invaginations are beginning to appear on the dorsal side, while 20*C* shows the first appearance of the ligule; and in this and the following sections the ventral invaginations are becoming conspicuous. Fig. 20*G* shows the transverse section of the mature leaf on a small scale; the mature phase has been described in detail by SABNIS (**25**), who has been so kind as to give me herbarium material for this study of the development.

SABNIS has also drawn attention to the fact that in *Panicum turgidum* and *P. antidotale* the limb is grooved on both surfaces (**25**). I have examined the developing leaves of *P. miliaceum* and *P. plicatum* (fig. 25*A*, *B*), both of which show the same character in their young stages. In *P. plicatum* the limb of the mature leaf opens out like a narrow fan from the top of the sheath (fig. 25*C*). DUVAL-JOUVE (**18**) has illustrated its structure. Fig. 25*D* is a section cut near the base of the limb, including part of the thickened midrib (*mr*). The plication appears to be brought about by intercalary growth between the main bundles.

In a previous paper (**4**) it was shown that invagination is one of the principal factors responsible for limb development in monocotyledonous leaves; the Gramineae may now be added to the list of families for which this generalization holds good.

Prophylls

The first leaf of the vegetative branch in the grasses, the so-called prophyll, is generally a two-keeled organ, with no bundle in the median plane. Two contrasting views have been held about its nature. While certain botanists have regarded it as a single leaf, whose peculiarities of form and structure are to be attributed to the conditions of pressure under which it develops, others, such as

GOEBEL (19) and REUTER (24), consider this organ to represent a pair of leaves congenitally fused, each keel corresponding to the midrib of one of the constituent members. BUGNON (13) concludes from an examination of *Dactylis glomerata*, that the prophyll in this species is a single leaf, reduced almost entirely to a sheath. He treats the bundle in that keel, which is alternate-distichous with the median bundle of the succeeding leaf, as the median bundle of the prophyll, and the bundle in the other keel as one of the main laterals. Apparently he proves his case conclusively for the species with which he deals; and in those prophylls belonging to other grasses which I have been able to examine, I have found confirmatory evidence for his view. A diagrammatic example is afforded by the section through the coleoptile and plumule of a seedling of *Triticum vulgare* (fig. 10*A*). There is a bud (b_1) in the axil of the coleoptile, and one of its two prophyllar bundles (*mb*), which is opposite to the median bundle (mb^1) of the next leaf, is distinctly larger than the bundle in the other keel. Moreover, the bud (b_2), which arises in the axil of the prophyll, occurs opposite to the bundle (*mb*), which seems to justify regarding *mb* as the median bundle, in a morphological sense, and thus in treating the prophyll as a single leaf. A bud in the axil of the coleoptile of *Hordeum vulgare* gave similar evidence (fig. 13*B*). It was highly embryonic, and only one bundle (*mb*) was differentiated, but this strand lay opposite to the position of the future median bundle of the succeeding leaf. In a similar bud from another barley seedling, bundles were visible in both keels, but the one opposite the median bundle of the next leaf was larger than the other. Fig. 8*B* shows a bud of *Coix Lacryma-Jobi*, in which the only prophyllar strand yet developed lies in the expected position for the median bundle, that is, opposite what will be the median bundle of L_1. The lateral bud of *Gracilea Royleana* (fig. 19) presents a similar case.

Fig. 15*A* represents a section of a lateral bud of *Zizania aquatica* from the axil of a plumular leaf. The prophyll bundles are not located exactly in the keels; in sections lower down in the same bud, the right hand bundle is farther out of the keel than in this figure. In a second bud in the axil of a younger leaf of the same seedling, only one bundle has appeared (that opposite the median bundle of the next leaf), and it lies, as in the case figured, out of

the keel. In the bud of *Gracilea Royleana* already mentioned (fig. 19), the main bundle of the prophyll was also observed to lie out of the keel. These cases seem to afford indirect confirmation of BUGNON's view, for, if the keels represent the midribs of two fused foliar members, one would expect the principal bundles to be placed exactly opposite to them. On the other hand, on the hypothesis that the keeling is merely the mechanical result of pressure exerted on a single foliar member during its development, there is no particular reason why the bundles should bear an invariable relation to the keels.

Fig. 12*B* shows a curious case in which a bud (*b*) in the axil of the coleoptile of *Hordeum vulgare* is connected by a bridge (marked with a cross) to the succeeding leaf (l_1); in other words, *b* and l_1 may be said to arise from a common rudiment. I have previously observed something not dissimilar in serial sections through the shoot of *Crocus speciosus* (2), in which the bases of the leaf rudiments show fusions, both among themselves and with the axis. Such examples seem to indicate, at least when one is dealing with the tightly packed organs in a monocotyledonous leaf bud, that there is some danger in drawing morphological conclusions from the discreteness or union of the rudiments from which these organs arise. One of the bud rudiments of *Coix Lacryma-Jobi*, whose history was followed, also behaved in an unexpected way. Fig. 8 *C–I* shows successive serial sections through this very young bud. For clearness the prophyll is left white, and the undifferentiated apical cone (*ap*) which succeeds it is dotted. It will be seen that as the bud is followed upward, it bifurcates. If it had been examined as a solid object, it would probably have been described as possessing a "*bifid prophyll*," but in reality, as these serial sections show, only one of the two points is formed from prophyll tissue, while the other represents the upward continuation of the bud axis, from which the next leaf is not yet detached. This case suggests that evidence relating to bifid leaf rudiments should be subjected to critical scrutiny before it is accepted.

Lower palea and cotyledon

In the recent work already mentioned (12, 13), BUGNON discusses and criticizes in some detail the views on the morphological nature

of the members composing the grass embryo given by Miss SARGANT and myself (**27**). He discusses both the interpretation of monocotyledonous seedlings in general, which is implicit in the paper in question, and also the special case of the grass seedling, with which it is more particularly concerned. It will be simplest to consider the general part of his criticism first. BUGNON regards the scutellum and coleoptile as together constituting a single seed leaf, comparable with one of the seed leaves of a dicotyledon, whereas, on Miss SARGANT'S theory of the monocotyledonous seedling (**26**), the cotyledonary structures of the grass (as of the other families in this class), represent the paired seed, leaves of the dicotyledon in a state of congenital fusion. This is a sharp conflict of opinion, and I wish to take this opportunity of saying that, although I accepted Miss SARGANT'S theory of the dual nature of the monocotyledonous seed leaf, both when our joint paper on the grasses was written and also at a later date (**1**), I have since concluded that her theory cannot be maintained, and that the seed leaf of the monocotyledon is, in reality as well as in appearance, a single foliar organ. I hope elsewhere to give my reasons for this change of opinion, which brings me into agreement with BUGNON in holding the scutellum and coleoptile of the grasses to constitute a single cotyledon. I should, however, join issue with him on the question of the nature of the epiblast, the little non-vascular outgrowth, which in some but not all grasses is found opposite to the attachment of the scutellum. For the case of *Avena sativa*, the external appearance of the epiblast (*bl*) is shown in fig. 5*B*, and its relation to the other parts of the seedling in fig. 14. Fig. 15 *B*, *C* represents transverse sections through the seedling of *Zizania aquatica* at and near the level of attachment of the epiblast, which in this species is unusually large. BUGNON regards the epiblast as a second cotyledon, whereas in our 1915 paper we spoke of it as merely an outgrowth "from the cotyledon or axis, or both, and of little morphological importance." The view then expressed I still hold to be substantially correct, so far as it goes, but I should like to define my present ideas about the epiblast more explicitly. I now think that CELAKOVSKÝ'S (**14**) interpretation of this organ is the nearest correct. His theory, which in recent years has been revived by WORSDELL (**28**), is that the epiblast represents the

"auricles" at the base of the leaf limb of such a plant as *Hordeum* (fig. 3*A*, *B*) in a state of congenital fusion. I should accept this view in its main lines, but with a certain modification. The epiblast may be visualized as equivalent to the auricles at the base of a petiolate "pseudo-lamina" such as that of *Sagittaria*, rather than the auricles of *Hordeum*, which are situated immediately above the top of the leaf sheath. This modification of CELAKOVSKÝ'S views is suggested because I consider that the epiblast, which is attached to the hypocotyl approximately opposite to the scutellum, does not occur at the right level to correspond to the auricles in *Hordeum*. This is a necessary corollary to the interpretation of the mesocotyl put forward in 1915, and which differs from that supported by CELAKOVSKÝ. I regard the scutellum, not as equivalent to the entire limb of such a grass as *Hordeum*, but rather as equivalent merely to the "blade" of such a leaf as the petiolate bamboo shown in fig. 2; while the stalk, corresponding to the region marked *pet* in this figure, is fused with the hypocotyl to form the mesocotyl. The true point of origin of the scutellum is thus at the first node, where the coleoptile is attached, but the stalk is sharply bent downward, and, instead of being free, adheres congenitally throughout its entire length to the axis. If the epiblast were related to the cotyledon exactly as the auricles of *Hordeum* are related to the foliage leaf, it would be found at the level of the coleoptile node, and not at the level of attachment of the free distal region of the scutellum. It may be noted that the succession of leaves in the seedling of *Victoria regia* shows that fusion of auricles is not an unprecedented event, and that such fusion may take place round an axis is demonstrated by the leaf of *Utricularia perfoliata* (20). Neither of these cases presents a perfectly exact analogy to the scutellum and epiblast of the grasses, but they may help to visualize the relations of those peculiar organs.

I am also unable to agree with BUGNON'S conclusions as to the morphological nature of the scutellum, mesocotyl, and coleoptile. I am in agreement when he compares the scutellum to the awn of the "inferior palea" of the inflorescence (fig. 4*a*), and the coleoptile to the distal part of the palea, above the insertion of the awn (fig. 4 *lig*), but not as to the relation of both cotyledon and inferior palea to the normal foliage leaves. I adhere to the old view that

the awn of the lower palea and the scutellum of the cotyledon are equivalent to the limb of the foliage leaf, while both the coleoptile and the part of the lower palea above the insertion of the awn are equivalent to the ligule of the foliage leaf. On the other hand, BUGNON regards the scutellum and coleoptile together, and also the palea and awn together, as representing the limb, alone, of the ordinary grass leaf. He interprets the coleoptile in the seedling, and the upper part of the palea, as arising from a pair of secondary growing points, lateral respectively to the scutellum and the awn. Such a view seems to raise quite unnecessary complications; it not only disconnects the organs in question from the other leaves of grasses, but also from monocotyledonous leaves in general. On the other hand, my view has the advantage of bringing the cotyledon and palea into line, not only with the foliage leaf of the grasses, but also with the cotyledon and foliage leaf of other monocotyledons, and even with certain foliar structures among dicotyledons. I should regard the awn of the lower palea in grasses (fig. 4*a*) and the scutellum of the grass cotyledon (*sc*, figs. 5*B*, 11*A*, 12*B*, 13*A*, 14, 15*B* and *C*), together with the part of the mesocotyl which represents its stalk, as corresponding with the solid limb of such a leaf as that of *Distichia* among the Juncaceae, or *Elegia* among the Restionaceae, or even with the "cornet" of the sepal of *Passiflora* (**6**; see also reference to HALLIER on p. 471). Further, I should regard all these organs as equivalent to the petiole of the dicotyledonous foliage leaf. It is true that in the grass cotyledon the coleoptile plays a more important part than the ligule in the foliage leaf, but this change in relative proportions is to be explained by correlative growth. The sheath region in the foliage leaf often attains great development, whereas in the seed leaf it is almost non-existent, and it is by no means unnatural that the ligule of the cotyledon should make up, by relatively excessive growth, for the atrophy of the basal sheath. We find a comparable case in the "stipula adnata" of *Potamogeton natans*, which reaches an extreme development, while the sheath region scarcely exists (**4**).

The chief new contribution in our 1915 interpretation of the grass seedling was the idea that the mesocotyl represents the fusion of the stalk of the scutellum with the hypocotyl. BUGNON opposes this view, and returns to the earlier theory that the mesocotyl

is merely an elongated node. It appears to me, however, that no theory of nodal elongation will explain the inverted position of the scutellum bundle in the mesocotyl (figs. 15*C*, *D*, *sc* b^1); but, on our fusion hypothesis, this curious orientation ceases to be surprising, and is, in fact, exactly what one would expect.

Summary

In the present paper the morphology and development of the leaves of the Gramineae are considered. BUGNON's criticism (**13**) is discussed, and it is concluded that, as in the case of the leaves of other monocotyledonous families, the grass leaf is best interpreted as a phyllode consisting of a sheathing leaf base and a petiolar limb. It is shown that the leaf of the Gramineae cannot be treated as an isolated type, but that there are striking parallelisms between the leaf of this family and of other monocotyledons; this parallelism comes out not only in the mature form, but also in the fact that invagination plays a conspicuous part in the ontogeny of the limb.

A study of the prophyll confirms the theory of BUGNON and others, that this organ is a single leaf, rather than a pair of foliar members in a state of congenital fusion.

BUGNON's recent criticisms of the views on the morphology of grass seedlings put forward in 1915 by Miss SARGANT and the writer are considered. He treats the scutellum and coleoptile as constituting a single cotyledon, and with this opinion the writer is now in agreement, although adhering in other respects to the views expressed in 1915. The question of the epiblast is discussed more fully than in the earlier paper.

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EXPLANATION OF PLATES XXVIII–XXX

Unless otherwise stated, all the drawings of sections are from microtome series through leaf buds, terminal or lateral, the series passing from below upward. Xylem marked in black; phloem, white; fibers, dotted.

PLATE XXVIII

FIG. 1.—*Lolium multiflorum:* foliage leaf, showing sheath (*sh*), ligule (*lig*), limb (*l*), axis (*ax*), nodes (*n*); ×0.5.

FIG. 2.—*Bambusa palmata* (*Arundinaria Kumasiana*): foliage leaf, with sheath (*sh*), of which part only is shown; ligule (*lig*), petiole (*pet*), pseudolamina (*ps. l*); ×0.5.

FIG. 3.—*A*, *Hordeum Zeocriton:* junction of limb (*l*) and sheath (*sh*), to show ligule (*lig*) and auricles (*aur*); about natural size; *B*, *Hordeum* sp.: diagrammatic sketch of one auricle (*aur*), with part of junction of sheath and limb, to show vascular supply of auricle (enlarged).

FIG. 4.—*Avena strigosa* (*Danthonia strigosa*): lower palea to show awn (*a*) (consisting of column, *c*, and "subule," *s*), and ligule (*lig*); slightly enlarged.

FIG. 5.—*Avena sativa: A*, seedling; *pl*, plumule; *col*, coleoptile; *c*, caryopsis; *r*, roots; ×0.5; *B*, base of seedling shown in *A*, with endosperm and fruit coat dissected away to show scutellum (*sc*), mesocotyl (*mes*), and epiblast (*bl*); slightly enlarged.

FIG. 6.*A*–*C*.—*Arrhenatherum avenaceum* var. *bulbosum:* transverse sections of leaf from apical bud; *A*, top of sheath, showing origin of ligule; *B*, origin of invaginations; *C*, limb; ×47.

FIG. 7*A*, *B*.—*Sorghum vulgare: A*, transverse section through coleoptile and plumular bud, showing origin of ligule in first leaf (l_1); *B*, l_1 at higher level; ×47.

FIG. 8*A*–*I*.—*Coix Lacryma-Jobi: A*, seedling about three weeks from sowing, from which sections shown in *B*–*I* were cut: *c*, caryopsis; *mes*, mesocotyl; l_1, l_2, plumular leaves; *r*, roots; ×0.5; *B*, transverse section of bud in axil of first plumular leaf (l_1) of seedling shown in *A* (×77); the axillant leaf would lie toward upper margin of page; *m b*, median bundle of prophyll (*pr*); *C*–*I*, successive transverse sections of bud from axil of l_2 in *A* (×193); prophyll is left white, and growing apex of bud is dotted; axillant leaf would lie toward upper margin of page.

FIG. 9.*A*–*C*.—*Arundinaria spathiflora:* three transverse sections through a leaf, from sheath (*A*) to limb (*C*); ×47.

PLATE XXIX

FIG. 10.*A*–*D*.—*Triticum vulgare: A*, transverse section through seedling just above first node (×47) to show, in axil of coleoptile (*col*) a bud (b_1) whose prophyll (*pr*), with median bundle (*m b*), has a bud (b_2) in its axil; l_1, first leaf of bud b_1, after prophyll; *m* b^1, median bundle of l^1; l_1, l_2, l_3, l_4, plumular leaves; *ap*, growing apex; *B*–*D*, sections from series through plumular leaf of another seedling (×77); *B*, sheath; *C*, origin of ligule; *D*, limb.

FIGS. 11–13.—*Hordeum vulgare:* fig. 11.*A*, transverse section of seedling, just above insertion of coleoptile (×23); *pr*, prophyll of bud in axil of first leaf (l_1); *sc*, scutellum; *ep*, epithelium; figs. 11*B*–*D*, further development of l_2; it will be seen from position of median bundle (*m* b_2) that sections drawn in *B*–*D* are placed at right angles to that drawn in fig. 11.*A*; fig. 12.*A*, seedling

(×0.5) from which section drawn in fig. 12*B* was cut; fig. 12*B*, transverse section through seedling just above insertion of coleoptile (but at lower level than fig. 11*A*) to show bud (*b*) in axil of coleoptile, connected by bridge marked with cross with first plumular leaf (l_1); scutellum shows columnar epithelium (*ep*), ventral scale (*v s*), and two bundles, ×23; fig. 13*A*, seedling from which section shown in fig. 13*B* was cut, drawn by Miss SARGANT, 13 days from sowing (×0.5); endosperm removed to show scutellum (*sc*); fig. 13*B*, transverse section of bud in axil of coleoptile (*col*) from seedling shown in fig. 13*A*; *m b*, only bundle differentiated in prophyll, *pr*; *col.b*, coleoptile bundle; ×77.

FIG. 14.—*Avena sativa:* transverse section through seedling, showing epiblast (*bl*), free on one side, and scutellum (*sc*), attached on other; *v s*, ventral scale of scutellum; *r*, roots; *ms*, mesocotylar stele; ×14.

FIG. 15*A*–*C*.—*Zizania aquatica: A*, transverse section of bud in axil of plumular leaf, which would lie toward upper margin of page (×77); *B* and *C*, transverse sections of seedling close to base of epiblast (*bl*), showing its attachment; scutellum, *sc*; scutellum bundle, *sc b*; scutellum bundle in mesocotyl, *sc* b^1; mesocotylar stele, *m s*; ×14.

FIG. 16*A*, *B*.—*Festuca* sp.: transverse hand sections of mature leaf (×14); *A*, sheath; *B*, limb.

FIG. 17*A*, *B*.—*Aira* sp.: transverse hand sections of mature leaf (×23); *A*, sheath; *B*, limb.

PLATE XXX

FIG. 18*A*–*C*.—*Nardus stricta:* three transverse sections through leaves of apical bud (×47); *A*, closed sheath of outer leaf; *B*, origin of ligule; *C*, limb.

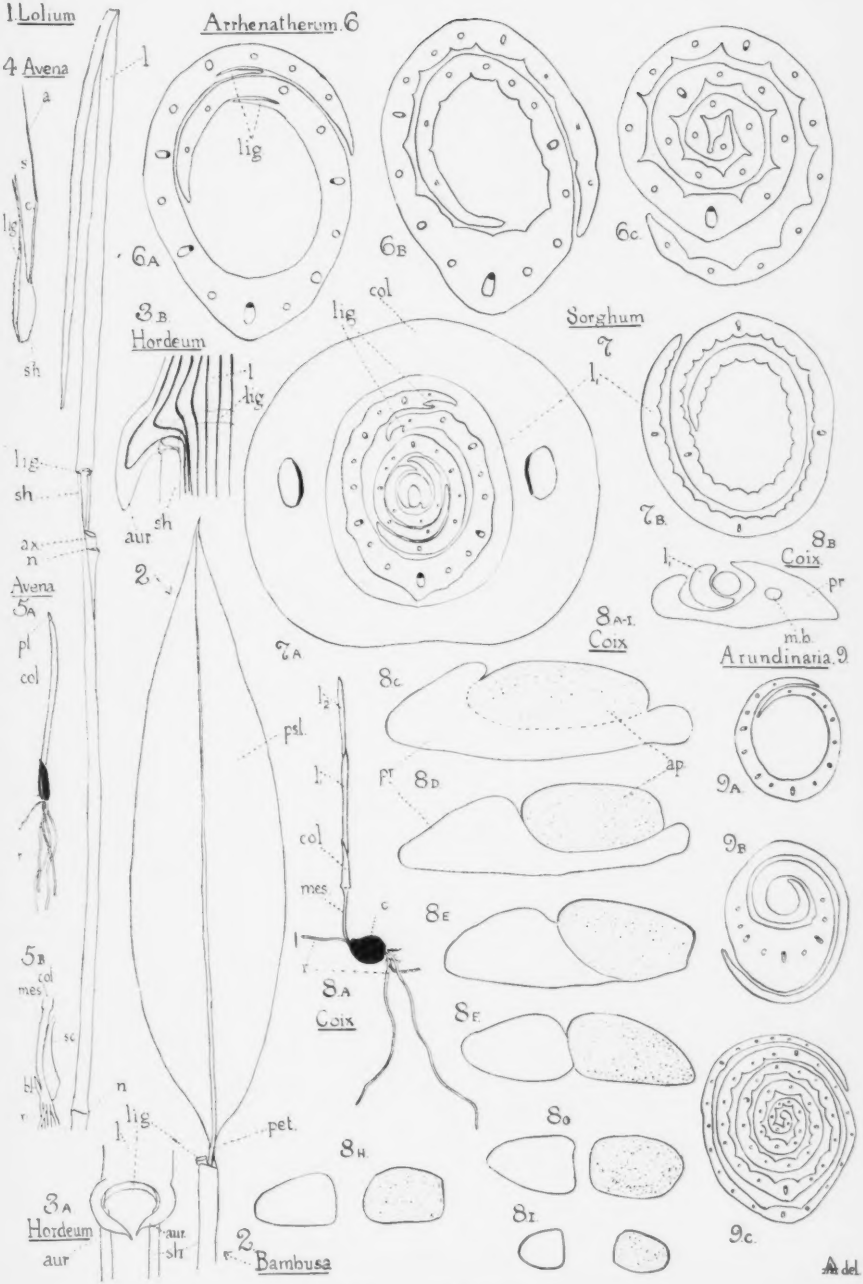
FIGS. 19, 20*A*–*G*.—*Gracilea Royleana* (Bombay Island): fig. 19, transverse section through apical bud (×77); outer leaf, each of whose bundles is accompanied by mass of fibers (*f*), is cut through its closed sheath, but one margin (*sh*) overlaps; *pr*, prophyll of bud; fig. 20*A*–*F*, series of transverse sections through leaf from bud (×77), passing from attachment to axis (*A*) through development and detachment of ligule (*C*–*E*) to limb (*F*); fig. 20*G*, transverse hand section of limb of mature leaf; ×14.

FIG. 21*A*–*C* and fig. 22.—*Ammophila arundinacea* (*Psamma arenaria*): fig. 21*A*–*C*, transverse sections from series through apical bud from Wells, Norfolk, to show origin of invaginations in leaf (l_2); fig. 22, transverse hand section of young leaf from another bud; ×47.

FIG. 23*A*–*C*.—*Lycium Spartum:* transverse hand section through sheath (*A*), base of limb (*B*), and higher in limb (*C*) of mature leaf; ×14.

FIG. 24*A*, *B*.—*Saccharum officinarum:* two transverse sections through three leaves (l_1, l_2, l_3) of apical bud, to show appearance of invaginations; ×23.

FIG. 25*A*–*D*.—*Panicum plicatum: A* and *B*, transverse sections of sheath and limb of developing leaf (×47); *C*, junction of sheath (*sh*) and plicate limb (*p l*) of mature leaf, showing ligule (*lig*) (×½); *D*, transverse hand section close to base of mature limb, showing part of thickened midrib (*mr*), and of one side of limb; *h*, hinge cells; ×14.



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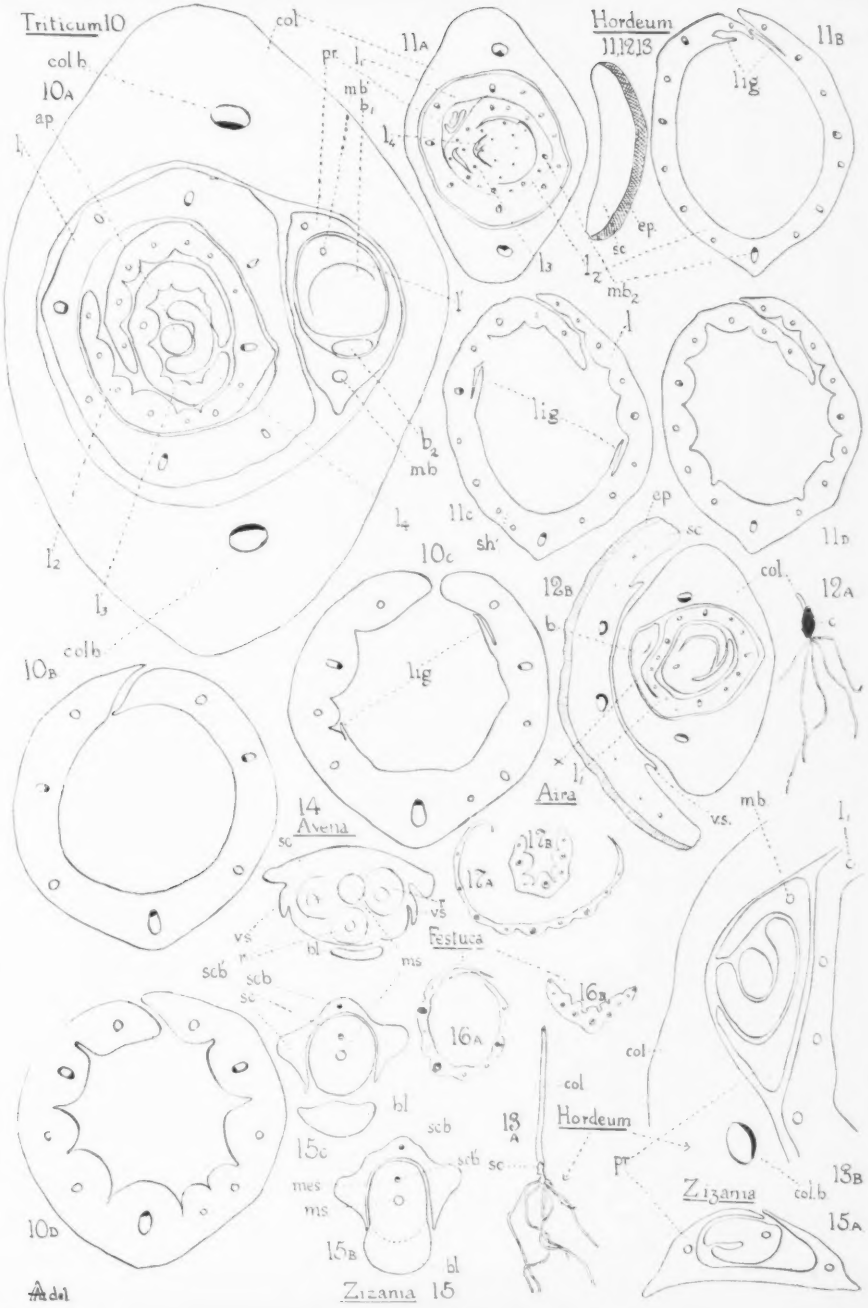

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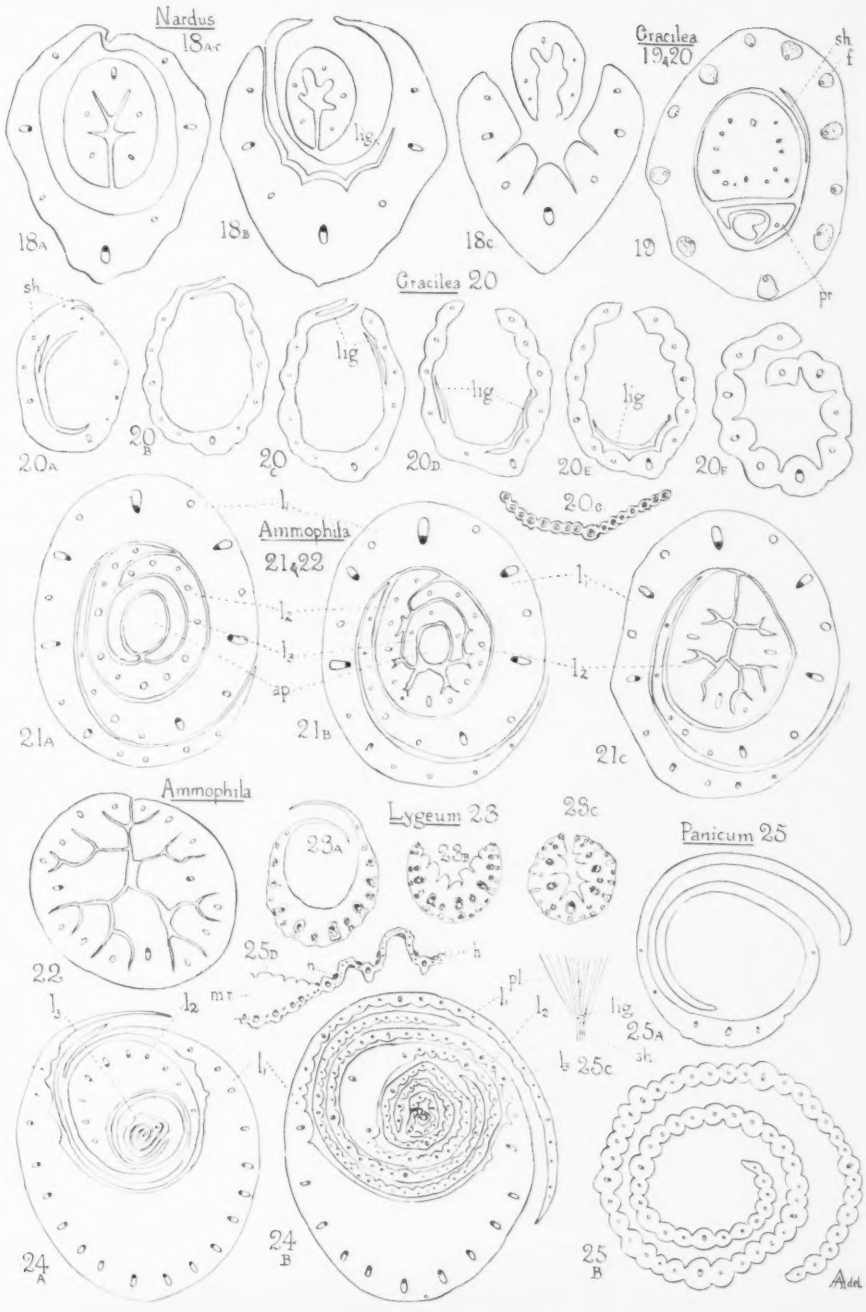

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REACTION OF PROTOPLASM TO SALTS AND ANTAGONISTIC ACTION OF SALTS AND ALCOHOL[1]

WILLIAM SEIFRIZ

Introduction

Some recent experiments by the writer on the reaction of the leaf cells of *Elodea* to certain reagents, revealed a striking difference in the toxic effects of the two bivalent cations, strontium and calcium, and a similarity in the effects of the monovalent cation sodium and the bivalent cation calcium. The changes in osmotic pressure and in protoplasmic streaming were qualitatively the same in the case of Na and Ca, but the exact opposite in the case of the two bivalent ions, Ca and Sr. Further, the change in osmotic pressure occasioned by Sr gave every indication of being due to an increase in permeability. These facts all stand in opposition to the widely accepted hypothesis that protoplasm reacts similarly to all monovalent cations on the one hand, and to all bivalent cations on the other hand, the former increasing, the latter decreasing permeability.

The experimental work on which this article is based was done in the laboratories of the Botanical Institute of the University of Geneva, Switzerland, where the writer enjoyed the privileges of a guest through the courtesy of the Director of the Institute, Professor R. CHODAT, to whom the writer's thanks are due for many kindnesses.

Method and material

The plasmolytic method of determining changes in osmotic pressure of the cell contents was followed. This method is not suitable for accurate quantitative measurements, but does give results which are often in harmony with those obtained by other methods, notably in regard to the order of absorption of different salts. A decided advantage of the plasmolytic method is the fact that the cells studied are under direct observation, so that any abnormal conditions, such as changes in surface tension, viscosity,

[1] Contribution from the Osborn Botanical Laboratory.

etc., which have an important bearing on permeability changes, can be detected.

The electrical conductivity method, while making quantitative measurements possible, is subject to criticism because of the impossibility of knowing whether the changes in resistance are actually due to corresponding changes in permeability. There exists the possibility that a change in conductivity may well result from a diffusion of substance from the salt solution into the tissue, bringing about changes in cell content of electrolytes without any change in permeability. That this may be true is indicated by the rapidity with which salts enter a cell in sufficient quantity to produce an immediate change in the relative positions of the cell inclusions. The writer has repeatedly observed a sudden aggregation of chloroplasts, before plasmolysis takes place, when a dilute solution of KNO_3 is added to an *Elodea* leaf. This immediate change in the position of the chloroplasts on the addition of a salt can only mean that there has been a very rapid entrance of the electrolyte into the cell, with no increase in permeability sufficiently great to permit a noticeable exosmosis of water. The whole problem of permeability presents so many difficulties that at present we can only present the data obtained from every possible method of attack. To restrict ourselves to any one theory or any single method of investigation is likely to lead to faulty conceptions.

Leaves of *Elodea* served as material. All observations were made on the superficial cells of the upper surface of the leaf. The reagents used were potassium nitrate (KNO_3), potassium chloride (KCl), sodium chloride(NaCl), calcium chloride ($CaCl_2$), barium chloride ($BaCl_2$), copper hydroxide ($Cu(OH)_2$), and ethyl alcohol (C_2H_6O). Spring water was used, the same in which the *Elodea* plants were kept growing in the laboratory, and which in Geneva is very pure.

I. Reaction of protoplasm to some monovalent and bivalent cations

Experimental data

Potassium nitrate was used as the plasmolyzing salt. The critical plasmolytic values were determined by ascertaining the

concentration of salt which would plasmolyze 50 per cent of the cells of a leaf in one-half hour. Owing to the rapidity with which the salt enters a normal protoplast, as is evident from the immediate aggregation of the chloroplasts, it was deemed best to shorten the time of application of the plasmolyzing salt as much as possible. The concentration of potassium nitrate which is isosmotic with the contents of the average normal epidermal cell of an *Elodea* leaf, that is, the critical plasmolytic concentration, is 0.3 M. Unless otherwise stated, this value is used for comparison. Since, however, the critical plasmolytic concentration of untreated cells varies considerably (it may be as low as 0.2 M and as high as 0.5 M KNO_3), it is highly important that the critical plasmolytic concentration of control leaves be determined in every experiment, and that these control leaves come from the same general region of the same shoot of *Elodea*.

Isosmotic concentrations of salts were used for treating the *Elodea* leaves in the case of potassium nitrate, sodium chloride, and calcium chloride. Lower concentrations of strontium chloride and barium chloride were ultimately employed, owing to the greater toxicity of these two salts. The following concentrations of solutions were used: KNO_3, 0.128 M, being the maximum concentration of this salt which will not plasmolyze a normal cell, no matter how long the time of treatment; NaCl, 0.128 M; $CaCl_2$, 0.128 M; $SrCl_2$, 0.064 M; and $BaCl_2$, 0.064 M.

Changes in osmotic value of cell.—Brief treatment, up to eighteen hours, in potassium nitrate, sodium chloride, and calcium chloride produces no change in the osmotic value of the cell which can consistently be determined by changes in the critical concentration of the plasmolyzing salt. Longer treatment in all three salts results in a gradual increase in osmotic pressure of the cell. This increase is much more pronounced in the case of the two monovalent ions, potassium and sodium, than it is in the case of the bivalent ion calcium. Two days in either potassium nitrate or sodium chloride will usually result in a critical plasmolytic concentration of 0.4 M or 0.5 M, as compared with a normal of 0.3 M. In calcium chloride prolonged treatment seldom results in a critical plasmolytic concentration of more than 0.4 M, and sometimes there

is no increase at all. Three days in potassium or sodium will result in an extraordinarily high critical plasmolytic concentration, which will reach 0.5 M for a few cells, 0.6 M for the majority, and over 1.0 M KNO_3 for some of the cells. The critical plasmolytic concentration of calcium treated cells never exceeds 0.5 M.

According to work on the permeability of protoplasm by the electrical conductivity method, one would expect the two bivalent elements strontium and barium to produce an effect on protoplasm similar to that produced by the bivalent element calcium, if, as is stated by OSTERHOUT (**6**), all bivalent cations (Ca, Ba, Sr, etc.) decrease permeability to a marked degree. Results obtained with the bivalent ions strontium and barium, however, are contrary to those of calcium. After twenty-four hours' treatment in 0.064 M $SrCl_2$, there is a slight reduction in critical concentration of salt. After forty-eight hours this reduction becomes pronounced, being then 0.2 M as compared with a normal of 0.3 M KNO_3.

Critical concentration values expressed in figures do not impress one with the change in osmotic value of the cell so forcibly as does a visual demonstration. If leaves treated for about two days (forty hours) in a 0.064 M $SrCl_2$ solution are plasmolyzed, together with untreated leaves, with 0.3 M KNO_3, and examined in half an hour, numerous cells will be slightly plasmolyzed in the control leaves, while in the strontium-treated leaves nearly every cell will be prominently plasmolyzed. Although these results were obtained in the great majority of cases, it is not always true that the decrease in osmotic value of strontium-treated leaves is so evident. Cells of different leaves, and especially of different lots of *Elodea* collected at different times and in different localities, vary greatly in their reaction to strontium.

The most startling reduction in critical plasmolytic concentration was observed in the case of barium chloride. A critical concentration value of plasmolyzing salt of 0.11 M was obtained with barium-treated cells, a value far below the average normal and considerably below the minimum value of 0.18 M ever found for a control leaf.

More highly toxic to protoplasm than any of the ions so far considered is copper. The copper ion was used in the form of

"oligodynamic" water. The term "oligodynamic" is NÄGELI's (**2**), and refers to water which has a very slight trace of copper. The slight trace of copper in water distilled in a copper still, or water in which copper coins have remained for a day or two, causes a very pronounced reduction in osmotic value of *Elodea* cells which are allowed to remain in the oligodynamic water over night. If an untreated leaf and a leaf which has been in the copper water for eighteen hours are plasmolyzed with 0.35 M KNO_3, within less than a minute all of the cells of the treated leaf (with the exception of the few which have been killed) will be slightly plasmolyzed, while none in the control leaf is plasmolyzed.

STIMULATION TO PROTOPLASMIC STREAMING.—Little streaming is to be observed in cells treated for a short time in potassium nitrate, sodium chloride, or calcium chloride. After prolonged treatment in these salts no streaming at all is seen. In strontium chloride, barium chloride, and oligodynamic water the stimulation to streaming is very great. Eighteen hours in a 0.064 M $SrCl_2$ solution is sufficient to arouse many cells to active streaming in a leaf reasonably sensitive to strontium. Two days will cause a great abundance of streaming, much of which is abnormal. In a leaf which had been in 0.128 M $SrCl_2$ for seventy-two hours (many cells succumb to so high a percentage of strontium for so long a period of treatment), the individual paths of flow in some cells were so numerous and so indefinite in their direction that all the chloroplasts seemed to wander about aimlessly. A very pronounced increase in the thickness of the protoplasmic layer, equaling five times that of the normal cell, resulted from treatment in strontium. The increase in thickness of the protoplasmic layer is probably the result of increased permeability of the protoplast as a whole to water and a resulting excessive imbibition. It is interesting to note that this highly swollen protoplast, undoubtedly in the gel state, continues very active streaming. Copper also has a very pronounced stimulating effect on protoplasmic streaming, even more so than strontium and barium.[2]

[2] A full discussion of the stimulation to streaming caused by strontium, barium, copper, alcohol, and saponin is given in a recently published article (**7**).

Discussion

If the cations potassium, sodium, calcium, strontium, barium, and copper are grouped on a basis of their effect upon the osmotic pressure of the cell sap and streaming of protoplasm, then potassium, sodium, and calcium fall into one class, and strontium, barium, and copper into another. Treatment of *Elodea* cells in potassium nitrate, sodium chloride, and calcium chloride results in an increase in osmotic value of the cell, as evidenced by the increase in critical concentration of the plasmolyzing salt. In contrast with the effect of the bivalent cation calcium on protoplasm is that of the bivalent cations strontium, barium, and copper.[3] As a result of treatment in the salts of these three elements, there is a surprisingly great decrease in osmotic value of the cells. Further, the bivalent cation calcium has no effect on protoplasmic streaming after short treatment, and puts an end to all streaming after long treatment, while strontium, barium, and copper cause a pronounced stimulation to streaming until death results from the high toxicity of these three elements.

There is every reason to regard the reduction in osmotic pressure of the cell, from treatment in strontium, as the result of increased permeability. Through a more porous membrane (using membrane in an abstract sense, since it is probable that the permeability of the protoplast as a whole is affected) an exosmosis of dissolved substances would take place which would lower the osmotic value of the cell. There is the possibility, of course, that the toxic substance might enter the cell and break down larger indiffusible molecules into smaller diffusible ones, and thus cause exosmosis without any change in permeability. The writer considered this possibility in the case of the reduction of osmotic pressure by senegin (a saponin), and was able to show by testing the resistance of the cells to alcohol, and on the basis of other observations as well, that decreased osmotic value of the cell in this case meant increased permeability (**8**).

The increase in osmotic pressure of the cell due to treatment in potassium, sodium, and calcium cannot so readily be interpreted in terms of permeability. That there has not been an increase in

[3] Copper in oligodynamic water probably exists as the hydroxide $Cu(OH)_2$, and is, as such, bivalent.

permeability is evident; otherwise exosmosis of the cell contents and a consequent decrease in osmotic value would have resulted. On the other hand, there is no reason to assume that increased osmotic pressure has been due to a decrease in permeability. The permeability might well remain unchanged, and the change in osmotic value be due to the toxic influence of the entering salt.

While the reaction of protoplasm to the bivalent cation calcium, in these plasmolytic experiments, cannot be interpreted in terms of permeability, it is quite clear that the bivalent cations strontium and barium cause a pronounced increase in permeability of the *Elodea* protoplast. This fact is in opposition to the statement of OSTERHOUT (**6**) that "all the bivalent kations" are able to decrease permeability "to a marked degree." This may be true of calcium, but, on the basis of these experiments, is not true of strontium and barium. It is possible that the difference in the results obtained with strontium by OSTERHOUT and the writer is due to the method of experimentation. It is also possible that the difference is due to the kind of material experimented upon. OSTERHOUT worked on marine plants (*Laminaria*). My experiments were done with a fresh water plant. The marked difference in salt content of sea water and river water would undoubtedly have a telling effect on the reaction which an aquatic growing in one or the other water would manifest as a result of treatment in salt solutions. FITTING (**1**), whose data stand in partial support of the writer's results, worked with the land plant *Tradescantia* (*Rhoeo*). He found that "salts for which the plasma has shown itself to be permeable, may, in concentrations approaching the critical plasmolytic concentration, diminish if not ultimately practically put an end to the permeability of the plasma membrane, long before the isosmotic value is reached, and whether the cells are plasmolyzed or not." FITTING worked primarily with KNO_3, but is of the opinion that other monovalent salts behave similarly.

Regardless of our interpretation of the experimental results in terms of permeability changes, one fact remains clear, that the reaction of protoplasm to the bivalent ion calcium is markedly different from the reaction of the living substance to the bivalent ions strontium and barium. It is quite evident, therefore, that while strontium, barium, and calcium possess certain physico-

chemical properties in common, and consequently can be grouped together chemically, they cannot be placed in the same biological class. This deduction can be stated in another way, that a plant shows the same discrimination in its reaction to ions as it does in its nutrition requirements.

Summary

1. Potassium, sodium, and calcium increase the osmotic value of the leaf cell of *Elodea*. The same three ions cause no stimulation to streaming after short treatment, and ultimately put a stop to all streaming.

2. Strontium, barium, and copper decrease the osmotic value of the *Elodea* leaf cell, owing to exosmosis due to an increase in permeability. These three ions cause a pronounced stimulation to protoplasmic streaming.

3. The results obtained by this plasmolytic method of experimentation indicate that elements which are of the same chemical class are not therefore necessarily of the same physiological class, as determined by their effect on protoplasm.

At the time that these experimental data were obtained, I fully appreciated that they were opposed to the widely accepted belief that monovalent cations such as Na produce an effect on permeability, coagulation, and like phenomena, which is opposite to that caused by bivalent cations such as Ca; and that two bivalent cations such as Ca and Sr each produce the same effect on protoplasm. The data were there, however, and no other interpretation was possible than that Na and Ca have an effect on protoplasm which is qualitatively the same, although differing quantitatively, while the effects of Ca and Sr are of opposite kinds. It was impossible to escape the conclusion that there is selective "permeability" in living cells, and that the valency hypothesis of the diffusion of ions through the protoplasmic "membrane" does not hold strictly.[4]

[4] After the manuscript of this article had been submitted for publication, an article appeared by LOEB (Jour. Gen. Physiol. 5:231–254. 1922) stating that "NaCl acts similarly to $CaCl_2$ and $LiCl_3$ on the rate of diffusion of acid into the egg of *Fundulus*." As Loeb states, it is thus necessary to regard the earlier experiments as incomplete, and to modify the valency hypothesis as applied to permeability. Certain

II. Antagonistic effect of salts on toxicity of alcohol

Experiments by LOEB and OSTERHOUT have shown that salts, such as sodium chloride and calcium chloride, which are toxic to protoplasm when alone, are not toxic when in combination. These two salts when in solution together represent a typical case of what is known as salt antagonism. According to the experimental work of OSTERHOUT, sodium chloride produces an increase in permeability, while calcium chloride produces a decrease. Consequently, when the two salts are together in proper proportion, that is, in a physiologically balanced solution, the normal diosmotic properties of protoplasm are not disturbed.

In some experiments on the reaction of protoplasm to alcohol (**8**), the writer found that ethyl alcohol in low concentrations is highly toxic to the protoplasm of the cells of the *Elodea* leaf, and that the osmotic value of these cells is greatly lowered by treatment in dilute ethyl alcohol, due to increased permeability and consequent exosmosis of cell contents. If sodium also increases permeability, as OSTERHOUT states, then one would rather expect it to add to the toxic effect of alcohol. Interpreted in terms of effect on osmotic value, as determined in these experiments, one would, on the contrary, expect sodium, which was found to raise osmotic pressure, to oppose the toxic effect of alcohol, since alcohol lowers the osmotic value of the cell. This latter assumption proved to be true. On the other hand, calcium, if it decreases permeability, should oppose the dispersing effect of alcohol. On the basis of effect on osmotic pressure, one would also expect antagonism between calcium and alcohol, but to a much less extent than in the case of sodium. In reality, calcium in solution with ethyl alcohol has no apparent effect whatever on the toxicity of alcohol.

European investigators have objected from the outset to the theory that "all the bivalent cations" are able to decrease permeability, while all monovalent cations (except H) increase permeability. The objections have been based in part on their own experiments and in part on lack of confidence in the electrical conductivity method of determining permeability changes. It is impossible to tell conclusively that the changes in conductivity observed by this method are actually due to changes in permeability. The selective permeability hypothesis of the early investigators apparently has come into its own again. The valency hypothesis, while receiving substantial support in many instances both in the non-living and the living world (for example, in "antagonism"), cannot be regarded as a theory of universal applicability.

In the majority of cases sodium chloride excellently prevented alcohol from exercising its toxic influence on protoplasm. To cite one convincing experiment, of twenty-five leaves placed in 10 per cent alcohol, and another twenty-five put in a solution of 10 per cent alcohol plus 0.128 M NaCl, and allowed to remain in these solutions for one hour the average number of cells killed in the alcohol was 86 per cent (maximum 98, minimum 75 per cent), while the average number killed in alcohol plus sodium chloride was 1 per cent (maximum 10 per cent in only one leaf, the next highest being 3 per cent, minimum 0 per cent). While these results were obtained in the greater number of instances, sodium chloride did not always so perfectly inhibit the toxic influence of alcohol. Occasionally the number of cells killed in a solution of alcohol and sodium chloride almost, although never quite, equaled the average number killed by alcohol alone. These experiments were repeated with calcium chloride instead of sodium chloride added to the alcohol. In not a single case was the number of cells killed in 10 per cent alcohol plus 0.128 M $CaCl_2$ much less than in alcohol alone, and frequently more.

The experimentation was carried further on the assumption that since calcium and sodium antagonize each other, then calcium and sodium both added to alcohol should antagonize each other and leave the alcohol free to exercise its full toxic effect on protoplasm. This assumption proved to be wrong. Leaves were placed in a solution of 10 per cent (2 M) C_2H_6O+0.128 M NaCl+0.128 M $CaCl_2$, and other leaves in 10 per cent alcohol, for one-half hour. The average number of cells killed in 10 per cent alcohol was 64 per cent. In the solution of alcohol plus the two salts, not a single cell succumbed, and this result was obtained in every instance of the many times that the experiment was performed. Sodium alone with alcohol varied considerably in its inhibitory powers. Sodium and calcium together successfully exercised their combined antagonistic effect on the toxicity of alcohol in every case. Potassium in the form of KNO_3 was substituted for sodium, and found to be as good an antagonizer as the latter; sometimes, like sodium, inhibiting the toxic effect of alcohol almost perfectly, and at other times showing little or no inhibitory influence.

The concentrations of sodium chloride and calcium chloride used with alcohol were the 0.128 M ones employed in the earlier experiments. While these concentrations of the two salts always prevented the death of all cells, yet all the cells were not always normal in appearance. With the idea that some other proportion might prove to be a more perfectly balanced solution, the quantity of calcium chloride was gradually reduced, keeping the amount of sodium chloride constant; and in another series the amount of sodium chloride was reduced, while the concentration of calcium chloride was kept constant. Two other solutions were prepared, in which the amounts of sodium chloride and calcium chloride in one solution were reduced to half, and in the other doubled. Of all these various combinations, only one proved to be less toxic than the original proportion. Doubling the amount of sodium chloride and calcium chloride (that is, 0.256 M NaCl+0.256 M $CaCl_2$) gave a combination of salts which more perfectly antagonized the alcohol, since not only were there no cells killed (when the percentage of cells killed in alcohol alone was 95 per cent), but all the cells were more normal in appearance, with only one apparent slight abnormality, namely an unusually high viscosity as evidenced by the irregular shape maintained by many of the plasmolyzed protoplasts.

Discussion

Three prominent facts stand out as a result of the foregoing experiments: first, calcium chloride never opposes the ill effect of ethyl alcohol on protoplasm; second, sodium chloride usually does act as an antagonizer, sometimes completely (so far as preventing death goes), but sometimes with very slight effect; and third, a combination of sodium chloride and calcium chloride acts unfailingly as an almost perfect inhibitor of the toxic influence of 10 per cent alcohol.

The effect of sodium on the toxicity of alcohol is in harmony, in part, with the other results obtained by the plasmolytic method. It was found (**9**) that alcohol lowers the critical plasmolytic concentration, and therefore decreases osmotic pressure (due to increase in permeability). It was further found that sodium chloride raises the critical concentration, at times to exceedingly

high values, and therefore increases osmotic pressure. A combination of these two substances should, and usually does, leave the protoplasm in a relatively undisturbed condition.

Calcium was found to be much less pronounced in its effect on the osmotic value of the protoplast than was sodium. One would expect, however, some effect from calcium as an inhibitor of the toxicity of alcohol. The fact that it does not antagonize alcohol while sodium does corresponds with the difference in valency of the two elements. It will be advisable to test the inhibitory effect of many other monovalent and bivalent ions on ethyl alcohol before arriving at any definite conclusions.

Similar cases of antagonism have been studied by other workers. TRAUBE (9) found that hemolysis of red blood corpuscles by NaCl was suppressed by isomyl alcohol. ZEHL (10) found that the sulphates of Co, Cu, Ni, and Al depress the poisonous action of isobutyl alcohol and an isomyl alcohol on the germinating power of fungus spores, but increase the toxicity of antipyrin, chloral hydrate, and chloroform. NOTHMANN-ZUCKERKANDL (3) found that NaCl causes no change in the toxic effect of ethyl alcohol (on *Echeveria* species), and materially increases the effect of propyl alcohol. $CaCl_2$ added to the toxic effect of propyl alcohol even more so than did NaCl.

Only a vast number of experiments will determine in what way the various salts and alcohols antagonize each other. The fact that NOTHMANN-ZUCKERKANDL found that NaCl has no influence on the toxicity of ethyl alcohol, while the writer found that it usually well inhibits the toxic effect of ethyl alcohol, is less disturbing than the fact that in my series of experiments, on one and the same plant, NaCl sometimes almost perfectly antagonizes alcohol, and sometimes exhibits little or no inhibitory action.

The word "antagonism" has been used in reference to the effect exercised by sodium chloride, and sodium chloride plus calcium chloride, on the toxicity of alcohol. The antagonism between sodium chloride and alcohol may not be such as that between sodium chloride and calcium chloride. The latter two salts are electrolytes, while alcohol is not. Antagonism between substances

in physiologically balanced solutions is not yet fully understood. The antagonistic action of salts was first thought to be "largely or entirely due to the fact that they hinder or prevent one another from entering the protoplasm" (4). This assumption was thought to be incompatible with the normal diosmotic functioning of the protoplast, and the theory of antagonism was changed. It is now believed that "salts antagonize each other because they produce opposite effects on the protoplasm" (5). While it is still possible that the original theory of antagonism is the correct one, namely, that the two substances in solution mutually hinder one another at the surface (or in the surface layer) of the protoplast, without totally preventing each other from entering in small quantities, yet between alcohol and sodium one is inclined to regard the antagonism as due to opposite effects of the alcohol and the salt on the protoplasm.

The variability in the behavior of sodium as an inhibitor of the toxicity of alcohol can only be explained on the assumption that it is due to a variability in the physiological state of the cells. The great variability in the resistance of cells to alcohol, where, for example, actively streaming cells are found next to dead ones, justifies such an assumption.

The behavior of sodium plus calcium in alcohol is equally puzzling. Calcium alone never inhibits the poisonous effect of alcohol, while sodium alone usually does, although it sometimes is as ineffective as calcium. Yet the two ions together always serve as almost perfect inhibitors of the toxicity of alcohol. It is possible that the sodium ion, which when alone usually prevents alcohol from causing death, is always able to exercise this inhibitory influence in the presence of the calcium ion. This suggestion is, in principle, supported by a very interesting hypothesis made to me by Professor CHODAT. It was suggested that, by virtue of Gibb's law, the Na and the Ca ions occupy different regions in the cell, whence the additive protective effect of the two when in solution together. If the monovalent ion is more in the periphery and the bivalent ion more toward the center, this would explain why the Ca ion when alone is unable to exercise any inhibitory influence.

Summary

1. Sodium chloride usually prevents ethyl alcohol from exercising its toxic influence on *Elodea* leaf cells.

2. Calcium chloride never antagonizes ethyl alcohol.

3. Sodium chloride and calcium chloride in combination always prevent ethyl alcohol from causing death in the *Elodea* cell.

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CYTOLOGICAL BEHAVIOR IN ROSA[1]

C. W. T. PENLAND

(WITH PLATES XXXI, XXXII)

Introduction

In 1916 a series of articles (**4–6, 13**) appeared from these laboratories relating to microspore formation as a criterion of hybridization in certain Rosaceae and Onagraceae. Previously and subsequently to that time numerous articles have been published in this and in other countries on the explanation of the polymorphism existing within these and other groups, and on its relation to mutation phenomena. For more than a century and a half, the abortion of pollen as a phenomenon connected with hybrids has been observed, and, according to BEER (**1**), "as long ago as 1850 WIMMEL called attention to the irregularities in the number and size of the pollen grains produced from the pollen mother cell of *Fuchsia*." *Rosa* has long afforded material for the study of similar irregularities. In 1884, CHRIST (**3**) observed:

> Chez ces Roses la faculté de former des hybrides est très prononcée . . . L'aspect de des Roses (cultivated hybrid roses) montre nettement qu'il existe chez elles une influence hybride.

In this preliminary paper it is hardly necessary to go into the question of imperfect microspore formation as a criterion of hybridization. The works of JEFFREY (**7–9**) and the others cited from this laboratory seem to carry proof on that point, and moreover cite voluminous literature, so that it will be needless to repeat those citations here.

This investigation is an attempt to discover the cause of polyspory by tracing the history of microspore formation, especially in the forms where polyspory is known to be present, and to explain this obvious irregularity. TÄCKHOLM (**14**), on cytological evidence established by himself, has demonstrated that the majority of European roses are of hybrid origin. At the same time, he indicates that most of the American species are sexually pure. This latter fact was a second point to be investigated in the present work.

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

BLACKBURN and HARRISON (2) likewise find that the British roses show cytological evidence of previous hybridization, and conclude that this is a method of microgene building. On the whole the results obtained so far seem to confirm the essential features of the papers of the European workers just cited. In view of the extent of the work and the time needed to complete it, it was deemed well to make a preliminary statement at this time.

Materials and methods

Rosa, besides being a large genus upon which to base conclusions, affords material admirably adapted to cytological study, because of the facility with which the buds may be collected and the size of the pollen mother cells. Work on this genus was begun in the spring of 1921, and at the same time work on certain other large genera of dicotyledons and of monocotyledons was undertaken in these laboratories by others. To date more than one hundred species, varieties, and hybrids have been collected from the Arnold Arboretum and the Botanic Garden of Harvard University. I wish here to thank the directors of these institutions for the permission of allowing these forms to be collected. Over one-half of the forms collected have now been studied.

In collecting, a number of buds of different sizes were taken, in order to be sure of getting the stages of division. The buds were fixed in chromoacetic. For imbedding, paraffin was used in the case of species with very small buds, but in the majority of cases the nitrocellulose method was employed. The former is advantageous when serial sections are desired, but in general the latter is a better method, and proves to be more economical in point of time. The use of Haidenhain's haematoxylin as a stain was found desirable, both because of the simplicity of application and the excellence of results. The sections were allowed to remain in iron alum for several hours, and then overstained in the haematoxylin and destained to the proper point in a weak solution of iron alum. Balsam was used for mounting.

Results and discussion

Although seven is the normal haploid chromosome number in *Rosa*, it was found that this number may be multiplied in certain

species, giving various combinations of the haploid number. The result of crosses between forms having different chromosome counts at once becomes a question of great interest and speculation. Among the diploid American roses are *R. blanda*, *R. Macounii*, and *R. gymnocarpa*. TÄCKHOLM finds *R. nitida* and *R. carolina* also to belong in this group. None of these has been suspected of spontaneous hybridization. Tetraploid roses are *R. pendulina*, *R. cinnamomea*, varieties of *R. spinosissima*, etc.; these all of Europe. Hexaploid forms are *R. acicularis* (Europe and America) and *R. nutkana* (America).

In addition to these roses which have chromosomes in multiples of seven, there are others having very diverse chromosome sets. *R. dumetorum* apparently is a pentaploid form, while *R. rubrifolia*, *R. Junzilli*, *R. alba*, *R. Gayiana*, *R. Harissoni*, *R. acicularis*×*rugosa*, and certain other garden hybrids have sets not yet determined. It will be seen that the majority of the roses in this and the last two groups above are European, some of course having American representatives (for example, *R. acicularis*, a very northern rose with us). Why hybridization should exist so abundantly among these last groups and their near relatives, and but scarcely among the first, can at least be conjectured. The esteem for the beauty of hybrid roses has probably led to their cultivation and hence dispersal in Europe, while in America long ages of isolation, previous to the discovery of the American continent, have given the roses a chance to become fixed. This hypothesis is by no means new, for it has been stated by many writers. It is true that certain European roses are credited with many hybrids. For example, CHRIST (3) credits *R. gallica*, the province rose, with thirteen.

At this point it will be well to take up the steps in the division of the pollen mother cell, which indicate the condition nearest normal in the case of certain roses. In all roses the stages in preparation for reduction, from the differentiation of the primary archesporial tissue, to chromosome formation in the stage of diakinesis, seem to be the same. For this reason only one figure (fig. 1) of the early stages has been shown. Here the chromatic nuclear thread is shown winding about within the nuclear membrane, and apparently inclosing the densely staining nucleolus, which

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commonly lies to one side of the nucleus. This is the stage just following synapsis, and just previous to the actual formation of chromosomes. Frequently congregations of chromatin grains may be observed, and it seems plausible to regard them as pro-chromosomes. (Note two of these at the upper right of the nucleolus).

Eventually the chromosomes become segregated and the diakinetic phase is entered. Here is first revealed the great diversity existing in the cytological behavior of different species of *Rosa*. Species which temporarily may be referred to as normal species, exhibit seven, fourteen, or a greater number of paired chromosomes (fig. 2). A multipolar spindle usually follows diakinesis, although it has not been observed in every case. The difficulty with which this stage can be found seems to indicate that the multipolar spindle is of relatively short duration, and of no great importance. Shortly the paired chromosomes arrange themselves on the equatorial plate with great regularity (figs. 3, 4). The anaphase (fig. 12) takes place also in a very regular manner, and soon gives rise to the telophase with the daughter groups of chromosomes at the poles. The second division is executed in a like regular manner, and eventually the tetrad is formed (figs. 7–9). In the forms with abnormal cytological behavior, a marked difference is to be found in the formation of the spindle and the resulting microspores. In the diakinesis an interesting situation is revealed. Here, besides a certain number of bivalent chromosomes, many so-called univalents are also found (fig. 14). Is this situation to be regarded as the addition of parental chromosomes, or the partial conjugation of unequal sets of chromosomes? Both may be true. In either case incompatibility, either quantitative or qualitative, is the probable explanation. As early as the anaphase it is apparent that the unpaired chromosomes have either lost their motility, or else are not subject to the same forces as the bivalents, for they are found at any conceivable place on the spindle, or may be found free from it and out in the cytoplasm (fig. 16). The majority of them, however, sooner or later become massed about the equatorial plate, following the bivalents, somewhat later, into the telophase (figs. 17, 18), thus giving an anaphase of two distinct steps. As

a result of this lagging, many of the univalent chromosomes become shut out of the membrane of the daughter nuclei. In subsequent divisions of the pollen mother cell they are found to produce separate spindles of their own. This situation has been observed and figured by JUEL (11)) and others, but without any attempt at interpretation. BEER finds in *Fuchsia* six, eight, and even ten microspores resulting from the same pollen mother cell, "due to the occurrence of irregularities in the distribution of the chromosomes during the anaphase." *Fuchsia* has long been subject to artificial hybridization in horticultural practice!

Throughout the remainder of division the irregularity becomes still more marked in these abnormal cytological forms, giving a situation just before the formation of the coat of the microspores similar to that shown in fig. 19. Here are seen numerous daughter nuclei, which are the products of more than the two normal spindles. Eventually a polysporous condition similar to that represented in fig. 20 is reached. Sometimes as high as ten or twelve microspores, or even more, come from the same pollen mother cell. The smaller of these pollen grains become the shriveled microspores in the adult pollen showing sterility. Indeed some of them appear to exist not even so long as this. Cases among certain garden hybrids are found where the whole anther collapses, apparently without the formation of microspores. Again, a few very large microspores may take the place of many normal sized ones.

Considerable attention is centered on the so-called univalent chromosomes. By some they are regarded as the result of constrictions of the bivalent chromosomes, but this does not constitute an explanation. Others hold that they represent physiological extrusion of chromatin material, the result of an attempt at genetical purification. Speculative as any explanation of their significance may be, their import in the mechanics of cell division cannot be underestimated; their presence is too suggestive. Whatever their phylogenetic or ontogenetic history, hybridism seems to be an influencing factor. The fate of these chromosomes is various; indeed, throughout meiosis they seem to be entirely subject to the laws of chance. Together they may form a dwarf microspore, or with a separate nuclear membrane be caught within the wall of a

larger microspore, giving a condition known as polycary (fig. 20). If the theory of the individuality of the chromosome is to be given credence, the significance of this irregularity in regard to mutation forms can readily be perceived. SAX (12) has shown that in *Triticum* the species with the greatest number of chromosomes were subject to greater variability and adaptation to economic use.

To say that this or that group of plants is excessively hybridized, is making a statement that calls for careful explanation. We find that hybridism, polyploidy, polyspory, and pollen sterility (10) are all related to each other as cause to effect in certain known plant crosses. Hybridization seems after all to be a question of degrees of removal of one parent from the other. Hence it is apposite to state here that this paper deals only with those forms where the removal is great enough to cause a disturbance in the critical phases of the reproductive cells. Polyspory almost invariably accompanies polyploidy if the stages of meiosis are irregular, and polyspory which eventually means sterile pollen is characteristic of many recognized hybrids. Hence when so many forms exhibit the meiotic irregularity characteristic of hybrids, it is but fair to conclude that they themselves are the products of comparatively recent heterozygosis.

In cases where polyploidy is absent, or at least not marked, four large microspores make up the completed tetrad, whereas in extreme polyploidous cases the members of the main tetrad are indistinguishable, and here almost complete sterility is found. No case has been observed where complete absence of extra-nuclear chromatin granules exists. In the more normal forms, however, they fail to produce well marked limiting membranes, and there is a conspicuous lack of karyolymph. This chromatin eventually becomes lost.

Conclusions

1. The normal haploid chromosome number in *Rosa* is seven.

2. Both orthoploid and anorthoploid forms based on this number are found.

3. Polyploidy is apparently due to the addition of incompatible parental chromosomes.

4. Polyspory is an accompanying result of polyploidy.

5. Several widespread American species of *Rosa* are characterized by relatively normal meiosis.

6. Proof of a physical basis for mutation seemingly presents itself in the supernumerary chromosomes.

I wish to thank Professor E. C. JEFFREY, under whom the work is being carried out, for kindly advice and assistance.

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EXPLANATION OF PLATES XXXI, XXXII

FIG. 1.—Prophase in *R. blanda*, showing nuclear skein and nucleolus.

FIG. 2.—Diakinesis in *R. blanda*.

FIG. 3.—Heterotypic spindle in *R. blanda*.

FIG. 4.—Equatorial plate in *R. Macounii*.

FIG. 5.—Telophase of heterotypic division in *R. gymnocarpa*.

FIG. 6.—Interphase following heterotypic division in *R. Macounii*.

FIG. 7.—Metaphase of homotypic division in *R. blanda*.

FIG. 8.—Completed tetrad in *R. blanda*.

FIG. 9.—Tetrad with separating microspores in *R. blanda*.

FIG. 10.—Multipolar spindle in *R. gymnocarpa*.

FIG. 11.—Equatorial plate in *R. pendulina*.

FIG. 12.—Heterotypic anaphase in *R. pendulina*.

FIG. 13.—Heterotypic metaphase in *R. nutkana*.

FIG. 14.—Diakinesis in *R. Kurdistena*.

FIG. 15.—Heterotypic telophase in *R. nutkana* with but few lagging chromosomes (?).

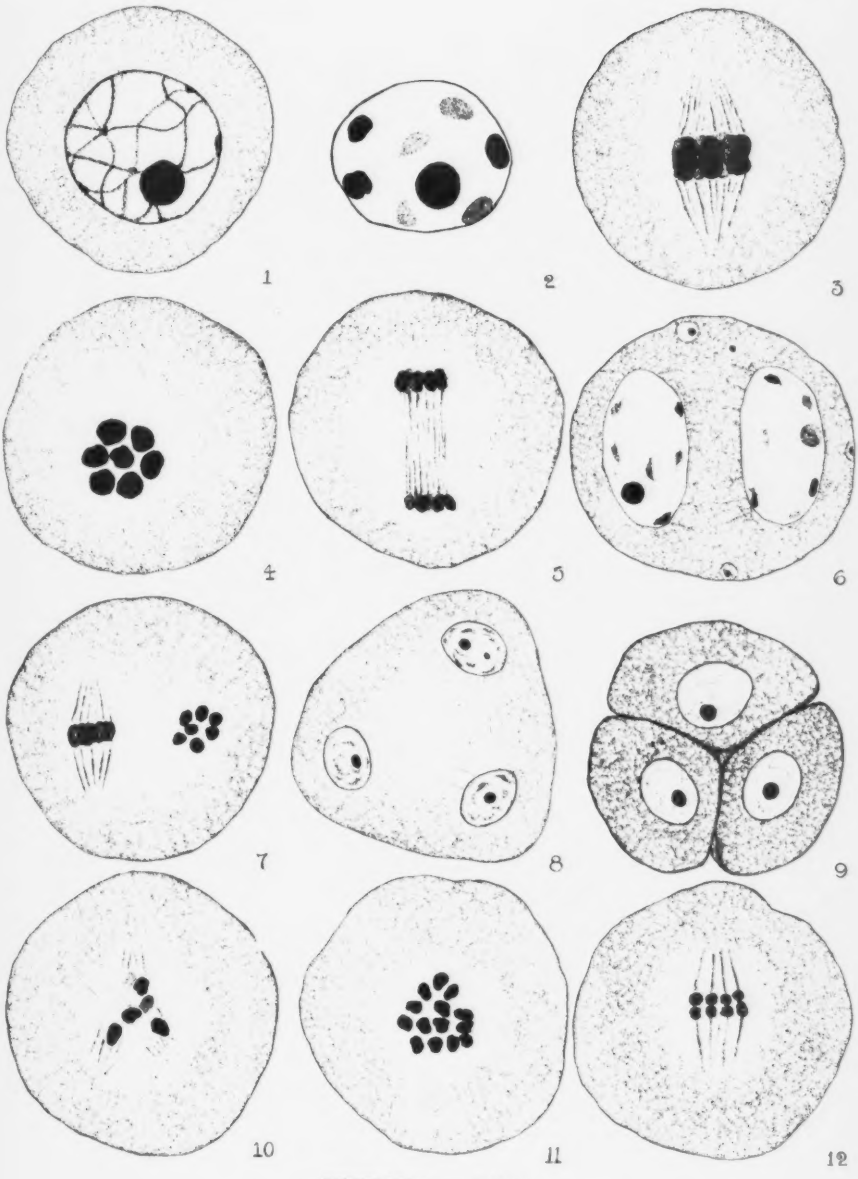
FIG. 16.—Heterotypic spindle in *R. dumetorum*, showing both univalent and bivalent chromosomes.

FIG. 17.—Heterotypic anaphase in *R. Gayiana* with univalents and bivalents in two distinct steps.

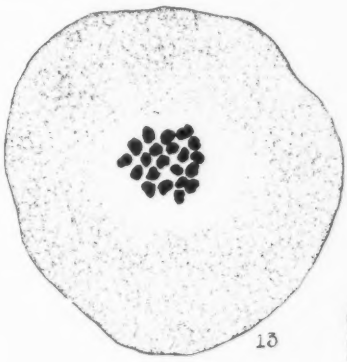
FIG. 18.—Same in polar view.

FIG. 19.—Completion of "tetrad" in *R. Alberti*.

FIG. 20.—Separating microspores in *R. rubrifolia*.



PENLAND on ROSA



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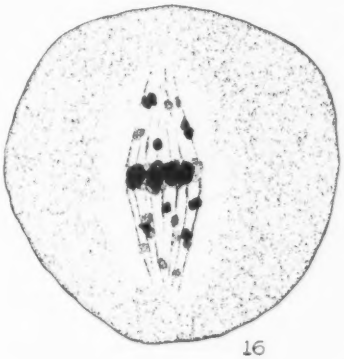

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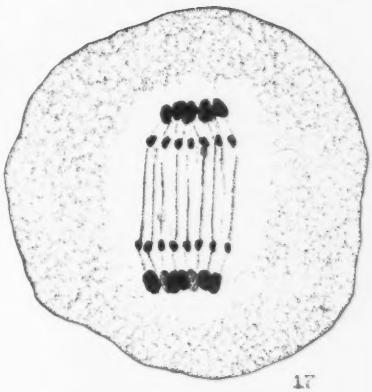

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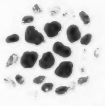

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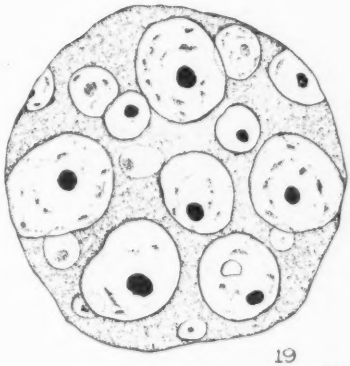

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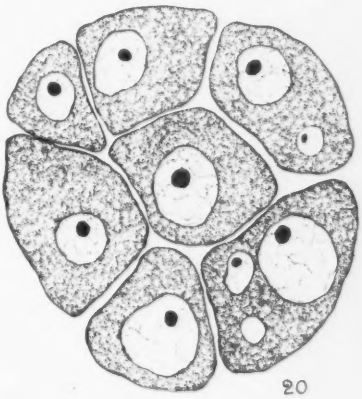

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PENLAND on ROSA

MATERNAL INHERITANCE OF CHLOROPHYLL IN MAIZE[1]

E. G. ANDERSON

(WITH TWO FIGURES)

A single maize plant having leaves conspicuously striped with pale green has given rise to a strain in which the pale green or striped character is transmitted only maternally. A preliminary report was read before the Society of American Naturalists at Chicago, December 1920. RANDOLPH (9) has described his cytological studies on the mitochondria and plastids of this strain, stating briefly the main characteristics of its inheritance. In all respects this case duplicates in maize the type of behavior which CORRENS (2) has observed in *Mirabilis*, BAUR (1) in *Antirrhinum*, and GREGORY (5) in *Primula*. The purpose of this paper is to present the genetic evidence bearing on the mode of transmission of this character.

ORIGIN.—The plant from which this strain originated appeared in 1918 in a large outcross family grown for pericarp color tests. It had leaves prominently striped with white or very pale green, while the remaining 120 plants of the same family were all normal green. The pistillate parent of the cross was Sanford's White selfed for one generation, and had been examined closely for traces of anthocyanin coloration in the leaves or stem. It is probable that even a very small amount of chlorophyll abnormality would have been observed. The staminate parent was the F_1 of a cross of orange pericarp by variegated. That this was the origin is checked by the facts that the striped plant had the same type of variegated pericarp as its sibs, and that when selfed it proved heterozygous for the "r^g" allelomorph which characterized the Sanford White parent.

PRELIMINARY TESTS.—This single striped plant was selfed and pollen from it was used on japonica, fine-stripe, and several normal green plants. The crosses gave only normal green F_1 progeny.

[1] Paper no. 105, Department of Plant Breeding, Cornell University, Ithaca, N.Y.

From the self-pollination a well matured ear of about 480 seeds was obtained. The first small planting of a few seeds from this ear gave two plants, both striped. A second planting, to determine seedling color, gave 3 green, 4 striped, and 21 entirely pale green seedlings. The pale green seedlings were lethal. A third planting was then made to determine whether the distribution of these types on the ear was random. Two rows from the ear were planted in order; one beginning at the shelled area and extending to the tip of the ear, the other a complete row on the opposite side of the ear. Of the former, the first 7 seeds gave pale green seedlings, the remaining 11 seeds toward the tip gave only normal green seedlings. The other row gave some pale and striped plants near the base and a few in a group just beyond the middle. The intermediate and the tip portion gave only normal green. The remaining seeds have since been planted, and the distribution of seedling chlorophyll color is shown in table I.

TESTS OF INHERITANCE.—*Progeny of green seedlings.*—The green seedlings were transplanted and remained green throughout their entire life. Self-pollinated ears were secured from 110 of these plants. Seedling tests of these ears gave over 25,000 normal green offspring, one albino, and one yellow. The two off-type plants appeared in the same culture and were probably due to a mutation to white in a plant heterozygous for the luteus factor (LINDSTROM **7, 8**). No pale green or striped seedlings were obtained.

Progeny of striped seedlings.—The pale striped seedlings were carefully transplanted and remained pale striped to maturity. One plant with only a narrow pale stripe produced only green leaves later and gave only green progeny. Those which were largely pale either died or grew slowly and failed to produce ears. Striped plants which produced ears were further tested. Some ears gave only green seedlings; others gave green, striped, and pale seedlings in various proportions. The source of pollen seemed to have no influence. Several plants were pollinated by mixtures of their own and foreign pollen. The foreign pollen was taken from varieties with yellow endosperm or purple aleurone, so that the crossed seeds could be detected by xenia. In areas where the selfed seeds gave pale seedlings the cross-pollinated seeds also gave pale seedlings.

TABLE I

DIAGRAM OF SELF-POLLINATED EAR FROM ORIGINAL STRIPED PLANT, GIVING APPROXIMATE POSITIONS OF SEEDS PRODUCING GREEN (Gr). STRIPED (*St*), AND PALE GREEN (Pa) SEEDLINGS; LARGE BLANK SPACE SHOWS AREA FROM WHICH SEEDS WERE SHELLED FOR FIRST AND SECOND PLANTINGS; OTHER SPACES INDICATE UNDEVELOPED OVARIES OR SEEDS WHICH DID NOT GERMINATE

Gr	Gr	Gr	Gr	Gr		Gr	Gr		Gr
Gr		Gr	Gr		Gr	Gr			
Gr	Gr	Gr	Gr	Gr	Gr		Gr	Gr	
Gr		Gr	Gr	Gr		Gr	Gr	Gr	Gr
Gr	Gr	Gr	Gr			Gr	Gr	Gr	Gr
Gr	Gr	Pa	Pa	Gr	Gr	Gr	Gr	Gr	Gr
Gr	Gr	*St*	*St*		Gr	Gr	Gr		Gr
Gr	Gr	*St*	Pa	*St*	Gr	Gr	Gr	Gr	Gr
Gr	Gr	Pa	Pa	*St*	Gr	Gr	Gr		Gr
Gr	Gr	*St*	Pa		Gr	Gr	Gr		Gr
Gr	Gr	Gr	Pa	Gr	Gr	Gr	Gr	Gr	Gr
	Gr	Pa	Pa	Pa	Gr	Gr	Gr	Gr	Gr
	Gr	Gr	*St*	Pa	Gr	Gr	Gr	Gr	Gr
Pa	Gr		Pa	*St*	Gr	Gr	Gr	Gr	Gr
Gr	Gr	Gr	Pa	Gr	Pa	Gr	Gr	Gr	Gr
St	Gr	Gr	Gr	*St*	Pa	Pa	Gr	Gr	Gr
Pa		Gr	Pa	*St*	Pa	Pa	Gr	Gr	Gr
Gr	Gr	Gr	*St*	Pa	Pa	Pa	Gr	Gr	Gr
Gr	Gr		Pa	Pa	Pa	Pa	Gr	Gr	Gr
Gr	Gr	*St*	Pa	*St*		Pa	Gr	Gr	Gr
	Gr	Pa	*St*	Pa	Pa	Pa	Gr	Gr	Gr
Gr	Gr	Gr	*St*		Pa	Pa	Gr	Gr	Gr
Gr	Gr	Pa				Pa	Gr	Gr	Gr
Gr	Pa	Gr	Gr				*St*	Gr	Gr
	Gr		*St*				Pa	Gr	Gr
Gr	Gr	*St*	Pa				*St*	*St*	Gr
Gr	Pa	Gr					Pa	Gr	Gr
Gr	Gr	*St*	Gr				Pa	Pa	Gr
Gr	Pa	Pa	Gr				Gr	*St*	Gr
Gr	Gr	*St*						Pa	Gr
Gr	Gr		Gr				*St*	*St*	Gr
Gr	Gr	Gr	Gr				*St*	*St*	Gr
Gr	Gr		Gr				Gr	Gr	Gr
St	*St*	Gr	Gr					Gr	Pa
Pa	*St*	Gr	Gr				*St*	*St*	*St*
St	Gr	*St*					Pa	*St*	Pa
Gr	Gr	Gr					Gr	Gr	Gr
St	Gr	Gr					*St*	*St*	
St	Gr	Gr						Pa	*St*
St	Gr	Pa						*St*	Pa

In areas where selfed seeds gave green seedlings, the crossed seeds behaved likewise. Seedling tests were made in flats, either with the position of each seed on the ear recorded, or with the plantings so made that the position of a seed in the flat would correspond with its position on the ear. It is not thought worth while to pub-

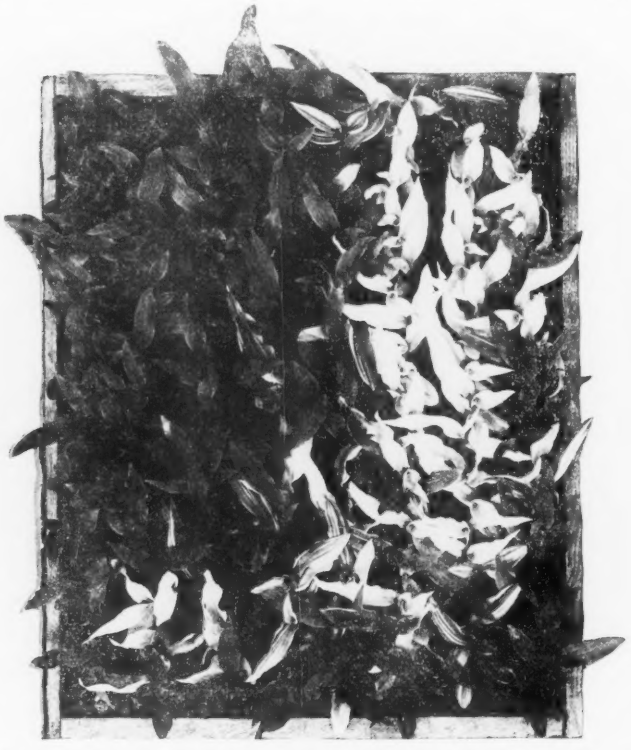

FIG. 1.—Progeny of striped plant; seeds planted so that position in flat would correspond to position on ear.

lish these distribution records. Fig. 1 is a flat showing the distribution of seedlings obtained from one ear. Fig. 2 shows parts of two full grown leaves of a striped plant. Striped seedlings from self and from cross-pollination were grown to maturity. They again gave progenies of green seedlings only, green, striped, and pale seedlings in various proportions, and in one case pale seedlings only.

TESTS OF POLLEN.—The crosses of unrelated normal green plants by pollen of the original striped plant were grown and selfed. Only green plants were obtained in both the first and the second genera-

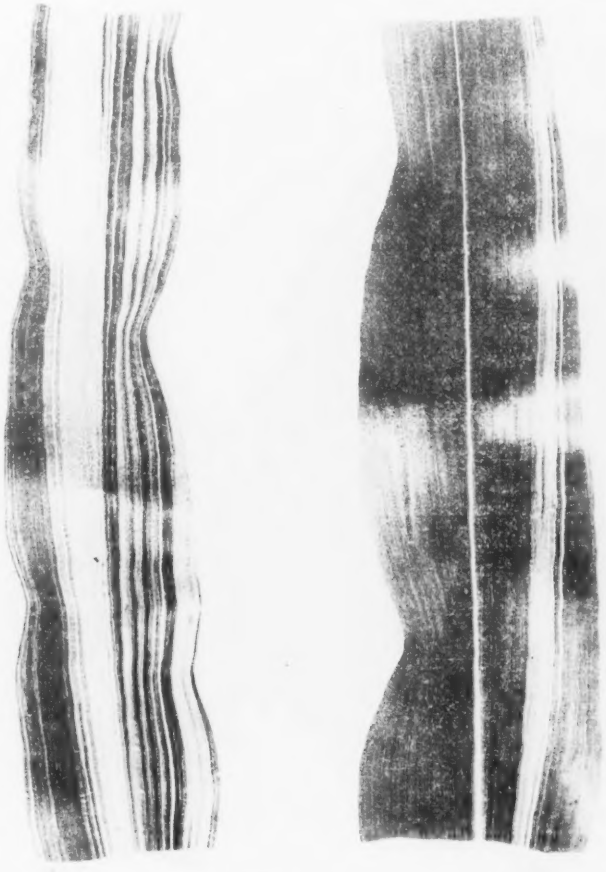

FIG. 2.—Leaves from striped plant

tion. Similar tests were made the following year, using pollen from four striped plants (selfed progeny of original striped plant). Only green plants were obtained in the first generation. In the

second generation, a few light colored seedlings appeared, which became wholly green after a few days' exposure to light. They were probably a form of "early virescent," a character which is widespread in maize, and is not detected except under closely controlled greenhouse planting. No pale or pale-striped plants appeared.

MECHANISM OF TRANSMISSION.—The transmission of the pale green character appears to be wholly maternal. The striped plants give green, striped, and pale green progeny regardless of source of pollen used. The distribution of these three types is not random, but large areas on the ears give only pale or only green seedlings. These areas seem to be those marked out by cell multiplication, and recall the mutation areas in variegated and mosaic pericarp (EMERSON **3, 4**; HAYES **6**). The striped plants are usually obtained from seeds on or near the borders of areas giving only pale or only green seedlings. Several Mendelian characters (yellow endosperm, pericarp color, and the *R* factor for aleurone and plant color) have been involved, and show random segregation throughout the entire ear. This indicates that the chromosome mechanism has not been disturbed.

The possibility of the character being due in some manner to an infectious organism is made improbable by the following facts: (1) There is no evidence of the character spreading to other portions of the plant than those marked out by cell multiplication. (2) During the four years in which this strain has been grown in the breeding plot, no repetition of this character has been observed in any other cultures. (3) The only means of transmission seems to be through striped plants. These occur as a very small percentage of the offspring of striped parents, and are usually so weak that the character can be perpetuated only with difficulty. But for the special care taken to perpetuate the character, it would probably not survive even for a generation or two.

CORRENS and BAUR each suggested that the cytoplasm of the egg was the basis for the transmission in similar cases in *Mirabilis* and *Antirrhinum*. GREGORY advocated the view that the plastids themselves are responsible, giving cytological evidence from *Primula*. This view is accepted by WINGE (**10**) for these cases

where segregation occurs into green, variegated, and white offspring. RANDOLPH, who has studied plastid formation in the present case, finds no visible difference between mitochondria and proplastids of normal and those of pale green tissue. Likewise, he finds no indication of segregation of the later stages or mature plastids into two classes, and is inclined to look elsewhere for the basis of the transmission of this chlorophyll character. The absence of anything other than plastid or chlorophyll characters behaving in this manner favors the view that the plastid primordia are the basis of transmission, but we still lack decisive evidence. At present, it is perhaps best to leave the question open, and to conclude only that the transmission is not by means of the chromosomes, but by means of some other cell organs, of which the proplastids or mitochondria seem the most probable.

Summary

1. A pale green striped plant appeared sporadically in a culture of maize free from chlorophyll abnormalities.

2. This chlorophyll character is transmitted maternally only.
   a. Striped plants self or cross-pollinated give green, striped, and pale seedlings in various proportions. The distribution of the different types is not random with respect to position on the ear.
   b. Green plants give green progeny only.
   c. Pale green seedlings die.
   d. Striped seedlings repeat performance of parent.

UNIVERSITY OF MICHIGAN

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EVAPORATION RATES ON ROCK CANYON WALLS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 313

FRANK THONE

(WITH ONE FIGURE)

Introduction

The vegetational succession on the walls of rock canyons has been discussed by COWLES (1). As the first barren furrow cut by a stream works its way downward, and increasing shelter gives rise to more favorable moisture conditions, a population of liverworts and mosses makes its appearance, clinging to the damp face of the rock. So long as the main cutting is vertical and the walls remain close together, affording mutual shelter, this moss mantle is fairly uniform in its floristic composition, but as the base level is approached and lateral cutting begins to widen the canyon, the upper parts of the walls become more exposed to drying influences, and the plants undergo a process of assorting according to their several abilities to withstand xerophytic conditions. They thus come to be arranged in a series of zones, ranging from the exceedingly moist mesophytic liverwort association at the base, through the somewhat more resistant mosses, to an upper zone of lichens in no wise different from the highly xerophytic initial stages of the rock successions of the open upland. The wider the canyon and the greater the exposure, the deeper does this upper zone of lichens (or even bare rock) become, crowding the moss and liverwort zones beneath it until these become only a matter of inches or even disappear altogether, unless some supplementary shelter, like a rock or a tree, protects a part of the wall from sun and wind.

Although these phenomena are well known, and the factors that govern them are well recognized, at present there is not on record any quantitative measurement of the factors in the water relations of plants as they exist on rock cliff faces. To be sure, the general principle is established that the greater the degree of exposure the higher the rate of evaporation; this has been

thoroughly developed by such workers as FULLER (3, 4), TRANSEAU (10), SHAW (7), and WEAVER (11). A recent paper by the writer (9) adds further data. Some work also has been done in the measurement of the increase in evaporating power of the air at different levels above the same point on the ground. YAPP (12) found that the evaporating power of the air at three levels in a marsh association (that is, on the ground, at one-half the general height of the plants, and above the tops of the plants respectively), stood in the ratio of 7:33:100. DACHNOWSKI (2) and SHERFF (8) in similar locations reached similar results. FULLER (5) measured the evaporating power of the air in three strata of a mesophytic forest. His three stations were 2 m. above the ground; immediately at ground level; and 4 m. below, at the bottom of a V-shaped ravine; and his mean daily evaporation rates for the season stood in the ratio of 184:100:80. A considerable variation in the evaporating power of the air with variation in elevation above the ground may therefore be expected.

The present problem

The data here presented were obtained in connection with those presented in a previous paper (9), during the months of July, August, and September, 1921, at the State Park at Starved Rock, Illinois. Four stations were established on the walls of Hennepin Canyon, one of the precipitous, U-shaped canyons characteristic of this region. Station 1 was located near the base of the east wall, close to the surface of a flourishing *Marchantia-Conocephalus* association. Station 2 was located about 3 m. above the canyon bottom, against the face of a well shaded portion of the rock covered with a growth of *Heterophyllon Holdanianum*. Station 3 was against the face of the cliff just below the level of the tree tops, thus in a position of moderate exposure. Station 4 was in the most exposed position it was possible to find on the cliff, well above the tree tops, and so situated that it received the full benefit of direct sunlight from early morning until late afternoon. Due to the continual crumbling of the soft sandstone of which the cliff is composed, there was little development of lichens, but the last two stations were otherwise in ideal locations for lichen growth, and may

legitimately be taken as representative types for lichen zone conditions. At the level of station 3 there were also several rock shelves supporting a growth of such plants as *Aspidium marginale*, *Polypodium vulgare*, *Pedicularis canadensis*, and *Campanula rotundifolia*. The data from this station, therefore, may be taken as representative of evaporation conditions for three different types of vegetation: the lichen zone, shelf plant associations, and the tops of the bottomland trees in the canyon.

Instruments and methods

Each station consisted of a radio-atmometric pair of standard Livingston spherical porous cups, equipped with the rainproofing valve described by LIVINGSTON and THONE (**6**). At station 1 the instruments were set on the floor of the canyon, and at stations 2, 3, and 4 they were mounted in special carriers which could be slung at the desired heights by means of cords and pulleys. During July readings were made every day, and from August 1 until September 20 they were made every two days; all readings were reduced to daily means for ten-day periods. It is a matter of regret that accidents prevented the beginning of observations before July 1. It is a matter of greater regret that no measurements could be taken of the water-supplying power of the substratum, but no method for obtaining a quantitative statement of the water-supplying power of a solid rock surface has as yet been suggested. It is necessary, therefore, to remain content with only half of the water relations, that of the evaporating power of the air.

Discussion

Fig. 1 indicates that we are dealing with the latter two-thirds of a seasonal transpiration march. The columns representing the evaporation rates at each station, whether for evaporating power of the air alone (black columns) or for the additive effect of direct insolation (white extensions), show high rates during July and the first ten-day period in August, with the climax during the mid-July drought, and a falling off after the oncoming of cooler weather during August. The flattening out of the curve during the last period in August and the first period in September is coincident with a short warm wave.

When a comparison is made between evaporation rates of the four stations for any given period, two points of outstanding interest present themselves. The first is the difference, predictable from a consideration of the studies mentioned in the introduction, between the rates for the lower and the higher stations. During

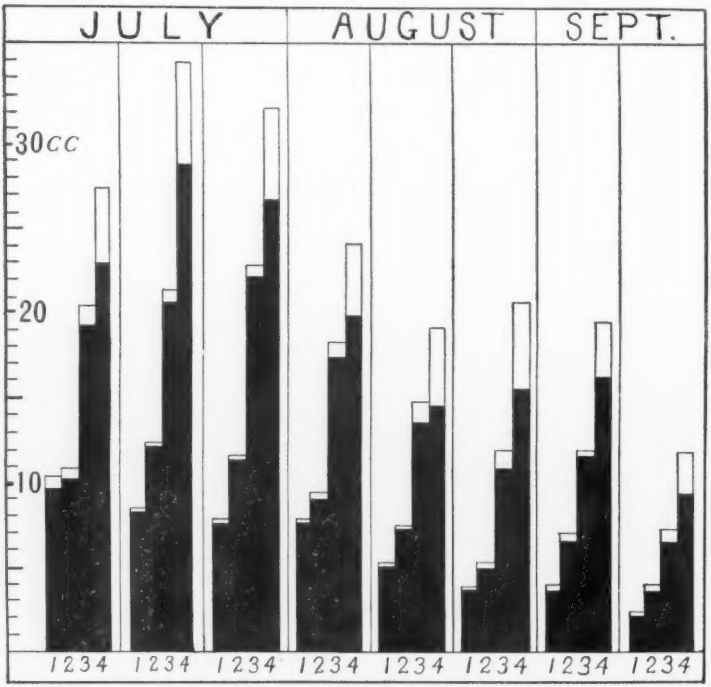

FIG. 1.—Mean daily evaporation rates from standard black and white spherical porous cup atmometers for ten-day periods, for four different heights on wall of sandstone canyon; black columns indicate evaporating power of air, white extensions increase due to direct insolation.

the period of highest evaporation rates (July 11–20) the ratio between the four stations, taken in ascending order, was 100:144:286:337. During the period of lowest evaporation rates (September 11–20) the ratio was 100:170:280:387. The highest station was thus at all times exposed to an evaporating power of the air between three and four times as great as that at the lowest

station. That the ratio between high and low stations increased toward the end of the season, when all rates were falling off, is not surprising; the atmometers at the lowest station were then evaporating almost no water at all. The comparative rates are thus more on the order of those obtained by FULLER in his forest stations than those of YAPP in the marsh. It must be recalled, however, that in the present study none of the atmometer sets was as well sheltered as those used by YAPP, inasmuch as two of his instruments were among the stems of a sedge association, whereas the writer's were necessarily all exposed.

The second point of interest is the relatively complete cutting off of the influence of insolation by the tops of the trees. The radio-atmometric effect at stations 1 and 2 is insignificant. The difference of a mere fraction of a cubic centimeter per day is, in fact, within the limits of possible error of instruments and manipulation, and for practical purposes might almost be regarded as non-existent. At station 3, however, there is a consistent radio-atmometric effect averaging about 1 cc. per day. It is impossible to say how much of this is due to the direct insolation to which this station was subjected for a short period about noon each day; at any rate, this station just under the tree top level does show an appreciable increase in evaporation rate due to sunlight. When, however, the rate for even this partly sheltered station is compared with that for station 4, the station of maximum exposure to the light, the modest increase in evaporation is overshadowed. During the period of greatest sunlight intensity (mid-July) the ratio of the radio-atmometric effect at station 4 to that at station 3 is about six to one, and even when the sun was well started on its southern retreat in September the ratio still remains three to one. The effect on the combined evaporating power of the air and direct insolation of this exposure above the level of the tree tops is so clearly shown in fig. 1 that no further comment is needed.

Summary

1. This study is of the evaporation rates at four different heights, representing increasing degrees of exposure, on the walls of a precipitous canyon in the Starved Rock, Illinois, region.

2. Observations covering a period between July 1 and September 20 showed a similar seasonal march in evaporation rates for all four stations, with the high point in mid-July and the low point in September.

3. As between individual stations, the evaporation rate at the highest and most exposed position was between three and four times that at the lowest and least exposed.

4. An appreciable increase in evaporation, due to insolation, was obtained only at the station located above the level of the tree tops.

The writer acknowledges the kind assistance and encouragement of Professors H. C. COWLES and G. D. FULLER.

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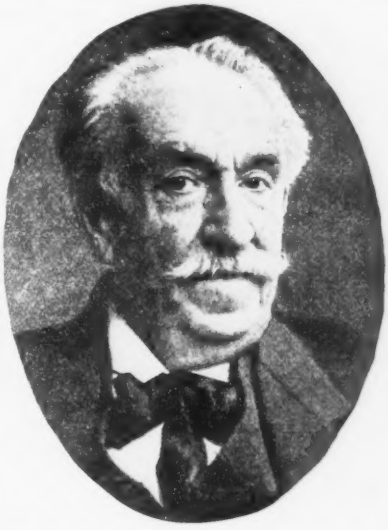
BRIEFER ARTICLES

GASTON BONNIER

(WITH PORTRAIT)

December 30, 1922, marks the passing of one of the most highly valued and respected figures in biological science, GASTON BONNIER. Born in 1853, this great French botanist carried on practically all his life work in and near the city of Paris. As a young man of twenty he went to l'Ecole Normale, and spent the next fourteen years on the faculty of that institution. His colleagues of those days attest BONNIER'S originality in the field of plant physiology, and tell how he opened for them an entirely new chapter, that of experimental morphology. He played a leading part in the organization of instruction in science, was instrumental in creating a special department of natural sciences, and in installing the first modern laboratories. In 1887 he took the chair of botany at the University of Paris, and within two years succeeded in establishing the famous forest laboratory of Fontainbleau. The quartocentennial of this event was celebrated in 1914 by a large group of his former students, at which time all joined in voicing their affection and admiration for their "Maître respecté."



Most of BONNIER'S investigations have been published in the Revue Générale de Botanique, a periodical which he founded and directed for thirty-four years. Not only did BONNIER carry on individual investigation of first rank, not only did he provide botanists with much in the way of valuable methods of investigation, not only did he make thou-

sands of students love the subject through his fascinating pedagogy, but also he made an enormous contribution through the part he played in the diffusion of the natural sciences before the general thinking public, with his many popular books and articles. Botanists all over the world will remember BONNIER for his classical experiments upon the structural changes induced by growing plants at various altitudes, experiments exemplary in their duration (over thirty years), and of profound significance for ecologists, geneticists, and many others. We feel assured that these very valuable experiments will be continued, and that the influence of BONNIER will be perpetuated in many other ways as well.—M. C. COULTER.

MULTIPLE PINE EMBRYOS

(WITH ONE FIGURE)

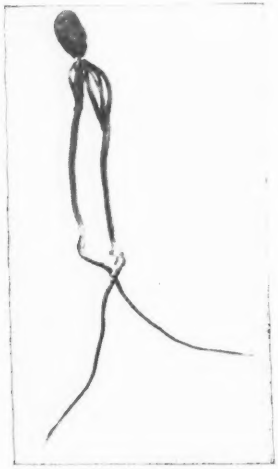


FIG. 1

Extensive germination tests on a large number of indigenous and exotic conifers have been conducted over a period of twenty years at the School of Forestry, Yale University. Germination tests made in April 1923 by LI TSI-TUNG on the seed of *Pinus Thunbergii* showed in one instance two well developed and normal seedlings from one seed. Heretofore, I have never encountered this condition in any of the conifers, and have been unable to find any reference showing that it has been observed before, although it has been recorded in *Ginkgo*. Fig. 1 shows the two seedlings natural size with the seed coats still attached.—J. W. TOUMEY, *Yale University, New Haven, Conn.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Physiology of germination.—A series of papers by HARRINGTON and others adds materially to our knowledge of the physiology of seeds and their germination. A new type of respirometer, suitable for medium sized objects, is described by HARRINGTON and CROCKER,[1] with details as to the method of use and calculation of results. This instrument has been used by HARRINGTON[2] in studying the respiratory intensity of dormant and germinating apple seeds. The respiratory quotient of dormant seeds at 10° C. is 0.70, which corresponds to complete oxidation of fats. Low temperature decreases the quotient, indicating oxygen storage, probably in organic acids and sugars. In advanced stages of germination the quotient may fall as low as 0.60, due to the transformation of fats to sugars and acids.

The after-ripening process of apple seeds has also been investigated by HARRINGTON and HITE.[3] The seeds are dormant at ripening of the fruit, because of a dormant embryo. After-ripening occurs in a few months at low temperatures if kept moist, or within the apple in cold storage. The optimum germination temperature for after-ripened seeds is between 10° and 20° C., and the seeds readily acquire secondary dormancy when kept under conditions that prevent germination.

Seeds of such grasses as Kentucky blue grass, Johnson grass, orchard grass, and Bermuda grass, and celery seeds germinate better in alternating than in constant temperatures.[4] For parsnip, celery, red top, and orchard grass, temperatures from 20° to 30° give good results, while Bermuda grass takes a higher range, 20°–35°, and Johnson grass does best at a very warm range, 30°–45° C. The high temperature should be used two to six or eight hours; the low temperature for the remainder of the 24-hour period. The alternations

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[1] HARRINGTON, G. T., and CROCKER, WM., A new and efficient respirometer for seeds and other small objects: directions for its use. Jour. Agric. Res. **23**:101–116. 1923.

[2] HARRINGTON, G. T., Respiration of apple seeds. Jour. Agric. Res. **23**:117–130. 1923.

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[4] HARRINGTON, G. T., Use of alternating temperatures in the germination of seeds. Jour. Agric. Res. **23**:295–332. 1923.

working best often correspond rather closely to the fluctuations of temperature occurring in soils at times that seeds germinate most readily.

Methods of forcing the germination of freshly harvested cereals like wheat, oats, and barley, which are dormant mainly from coat characters, have been worked out.[5] Artificial drying of the grain, breaking the coats, treatment with sulphuric acid, increased oxygen pressure, presoaking, and low temperatures, all have a certain influence in hastening germination tests. Apparently the treatments center mainly about the oxygen supply, as most of these factors are effective because they increase the oxygen supply by removing coat structures, or by increasing coat permeability to oxygen.

The best contribution of the series deals with the physiology of Johnson grass seeds.[6] Johnson grass is quite dormant just after maturity, and naturally requires a long period of after-ripening. The hard tight glumes are partly responsible for the delayed germination, but the pericarp and inner integument are mainly responsible for the dormancy. The structure of these coverings is considered in detail. The layers of the pericarp contain tannin compounds, and some layers are suberized, others impregnated with lipoids. These compounds may diminish the extensibility of the membranes, and limit imbibitional water intake sufficiently to prevent germination. They may decrease also the permeability of the investing membranes to solutes, and possibly to internal inhibitory substances which maintain the dormancy of the embryo. Effective forcing agents may oxidize, or precipitate the inhibitors, or change the permeability so that they can diffuse out. Chemical treatments may act either by reducing the resistance of the coats to imbibition pressures, or by directly stimulating the protoplasm. The whole series of papers constitutes a valuable contribution to seed physiology.—C. A. SHULL.

Epigaea and a soil fungus.—COUNCILMAN[7] has discovered that the roots of *Epigaea repens* are constantly associated with a fungus, and concludes that the relation is one of "perfect symbiosis," each serving the other in certain ways that are suggested. Examination of herbarium material from northern Japan showed the same relationship, including even the intracellular growth. The author remarks that "this goes to show that the relation of fungus to plant prevailed in the plants at their common source before the last glacial period."—J. M. C.

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[7] COUNCILMAN, W. T., The root system of *Epigaea repens* and its relation to the fungi of the humus. Proc. Nat. Acad. Sci. **9**:279–285. *figs. 4.* 1923.

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